



Redescription of larva, pupa and imago male of *Chironomus* (*Chironomus*) *salinarius* Kieffer from the saline rivers of the Lake Elton basin (Russia), its karyotype and ecology

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Abstract

Cytology and ecology of *Chironomus* (*Chironomus*) *salinarius* Kieffer, 1915 (Diptera, Chironomidae) was examined from material collected in the saline rivers of the Lake Elton basin (Volgograd region, Russia). Larvae of *salinarius*-type were identified as *C. salinarius* on the basis of their karyotype. The species is redescribed on the basis of all metamorphic stages. The reared imago and karyotype were obtained from larvae of the same population. The karyotype of *C. salinarius*, detailed mapping of the 5 chromosome arms A, C, D, E, F and characteristics of chromosome polymorphism are provided. Information on distribution and ecology of *C. salinarius* from the saline rivers (total mineralization 6.8–31.6 g l⁻¹) of the Lake Elton basin is also given. *Chironomus salinarius* is a common in the saline rivers and occurs in sediments with high silt content. On the basis of recent samplings *C. salinarius* appears to be very abundant in saline, mesotrophic as well as in eutrophic rivers. *Chironomus salinarius* accounted for 49–66% of total abundance of zoobenthos in water with salinity up to 13–31.6 g l⁻¹.

Key words: *Chironomus salinarius*, non-biting midge, taxonomy, karyotype, inversion polymorphism, saline rivers, environmental factors

Introduction

Chironomus (*Chironomus*) *salinarius* Kieffer, 1915 is a very important component of inland waters. The larvae of *C. salinarius* are a common species of brackish water such as saline rivers of the Lake Elton basin. These rivers are characterized by a large range of salinity. This indicates that *C. salinarius* can tolerate important variations of salinity.

The imago male and female of *C. salinarius* were described by Kieffer (1915). Then Karl Strenzke (1959) in the article devoted to the revision of the genus *Chironomus* Meigen from the Thienemann's collection established a new type and gave a more detailed illustrated redescription of the male and female. Linevich & Erbaeva (1971) studied for the first time all phases of the metamorphosis rearing from larvae to adults. Sasa (1978, 1996) made a brief taxonomic description of imago male. *Chironomus salinarius* was reported for the first time in Korea by Chun (1989) in his Master of Science thesis. He gave brief descriptions of the larva, pupa and male imago. Later Ree & Yum (2006) redescribed this species from Korea on the basis of the imago male morphology. All the above mentioned papers contained brief and insufficient descriptions and did not include karyological studies. It is a matter of fact that karyological methods have given the most accurate and reliable results in identification of chironomid species on larval stage, while many species are practically indistinguishable on the basis of morphological characters. In this work the larvae of *salinarius*-type were identified as *C. salinarius* on the basis of

their karyotype. We present the redescription of the imago males, pupae and larvae on the basis of rearing larvae to imago males.

The karyotype of *C. salinarius* was described for the first time by Keyl & Keyl (1959), but the cytocomplex membership of this species had an unclear position in Keyl (1962). Further information about the *C. salinarius* karyotype and chromosome polymorphism has been also reported by Michailova (1973, 1980, 1989) and Grinchuk (1979, 1984). Some difficulties in studying *C. salinarius* karyotype are connected with a designation of chromosome arms. These difficulties were considered in detail in previous paper (Istomina *et al.* 2012). This paper provides a description of the karyotype, detailed mapping of arms of the 5 chromosomes A, C, D, E, F and characteristics of *C. salinarius* chromosome polymorphism with use of the arms designation and mapping system introduced by Keyl (1962), Dévai *et al.* (1989), Kiknadze *et al.* (1996).

The distribution of *C. salinarius* in the saline rivers of Russia (Volgograd region) has not been studied up to now. Many migratory birds use saline rivers of the basin Lake Elton as stopover sites. Migratory birds selectively consumed larvae of *C. salinarius* among other potential prey, which were abundant and readily available (Sanchez *et al.* 2006; Estrella & Masero 2010). The larvae represented up to ~97% of the total number of captured prey of water birds (Estrella & Masero 2010). Thus, data on ecological traits of this species, including distribution, limits of tolerance and diet peculiarities, is very important for environmental management. However, the insects in general and the chironomids in particular are neglected groups in this respect. In this paper we give details information on distribution and ecology of *C. salinarius* in the saline rivers of Volgograd region.

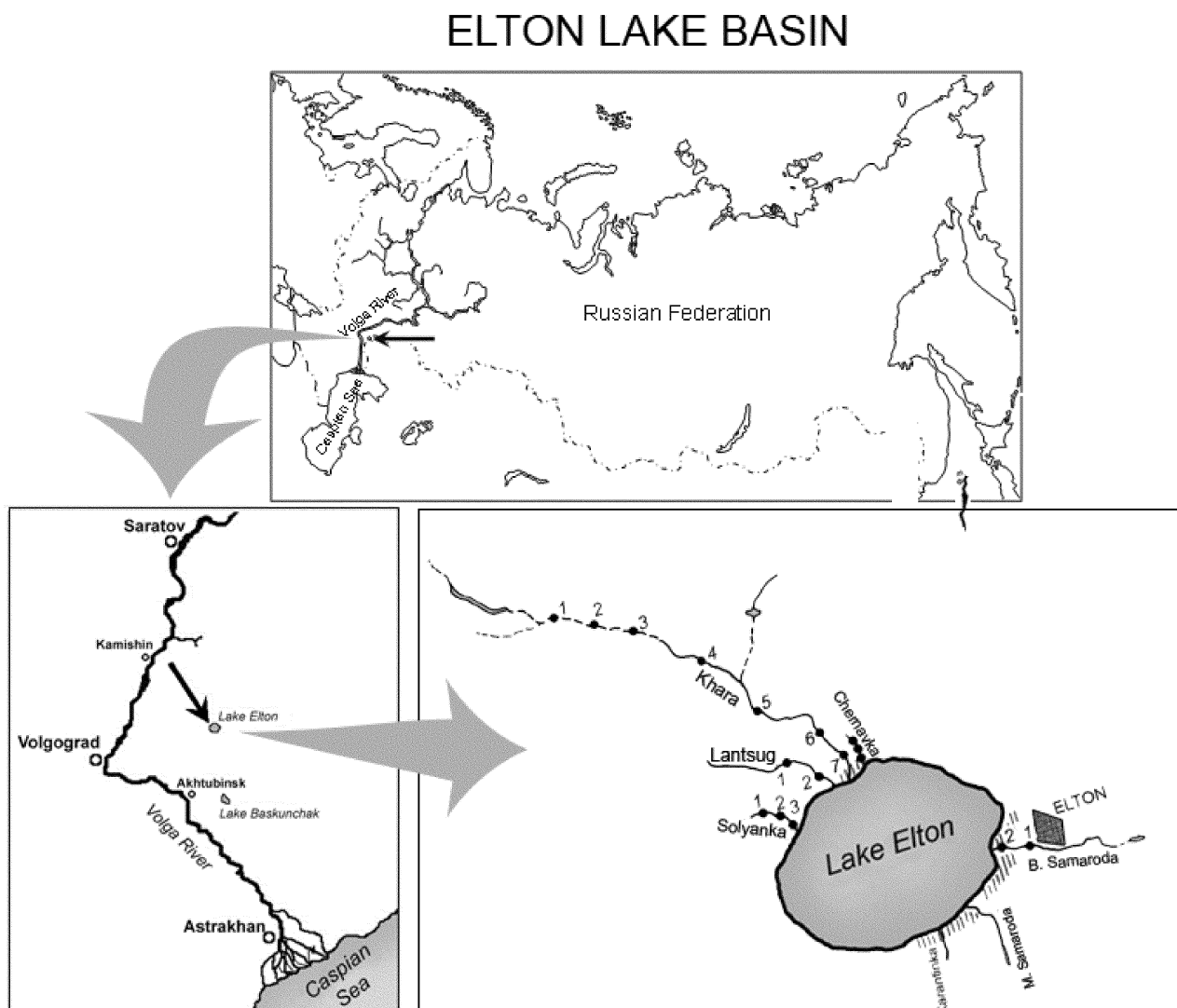


FIGURE 1. Map of the study area indicating sampling sites.

TABLE 1. Physical, chemical^a parameters (min-max) measured at habitats of larvae of *C. salinarius* in the rivers of the Elton Lake basin (August 2007–2012, July 2011), (April 2007, May 2011, 2012), (September 2008).

Physical, chemical parameters	Rivers						
	Lantsug	Khara	Chernavka	Solyanka	Bolshaya Samoroda	Malaya Samoroda	Karantinka
pH	7.3–8.6	7.2–10.0	7.2–8.3	7.6–8.4	7.4–8.4	7.9–8.2	7.6–8.2
O ₂ (mg l ⁻¹)	4.8–44.4	4.3–25.5	10.4–31.6	8.3–25.0	9.2–16.4	4.3–6.7	4.7–19.3
Temperature (°C)	14.8–26.0	12.0–32.0	12.5–31.5	21.3–30.2	12.3–31.1	26.0–27.3	22.7–27.0
Salinity (g l ⁻¹)	7.5–30.0	8.6–21.1	17.2–31.6	25.0–28.6	9.6–10.3	41.1	12.1–13.7
Na ⁺ +K ⁺ (g l ⁻¹)	2.11–9.07	2.39–6.09	3.43–9.63	7.76–8.86	2.68–2.73	7.89	2.76–3.97
Ca ²⁺ (g l ⁻¹)	0.28–0.80	0.26–0.88	0.88–1.20	0.88–1.22	0.40–0.56	2.00	0.42–1.00
Mg ²⁺ (g l ⁻¹)	0.17–1.17	0.15–0.92	0.68–1.22	0.57–0.76	0.16–0.41	3.53	0.45–0.49
Cl ⁻ (g l ⁻¹)	2.61–18.64	2.09–8.52	10.24–18.64	15.13–17.38	4.18–4.52	21.57	6.52–6.61
SO ₄ ²⁻ (g l ⁻¹)	1.69–2.99	2.03–8.33	0.56–0.96	0.14–0.39	1.64–1.70	5.58	0.91–1.91
HCO ₃ ⁻ (g l ⁻¹)	0.17–0.38	0.02–0.60	0.21–0.32	0.09–0.34	0.34–0.45	0.43	0.29–0.46
NH ₄ -N (g l ⁻¹)	0.8–25.1	0.7–24.7	7.0–49.2	3.1–69.0	1.2–2.9	*	4.3–6.4
NO ₃ -N (mg l ⁻¹)	0.01–1.00	0.09–1.10	0.09–0.14	0.09–0.10	0.09–0.10	*	0.09
NO ₂ -N (mg l ⁻¹)	0.01–2.25	0.01–1.61	0.26–0.84	0.19–0.65	0.01–0.24	*	0.19–0.27
Total P (mgP l ⁻¹)	0.14–0.28	0.12–1.93	0.24–0.25	0.19–0.42	0.93–3.18	1.17–3.0	2.75

Notes: *parameter was not measured; ^a The data was kindly provided by Center of Monitoring of Aquatic and Geological Environment, Ltd. (Samara, Russia), which has Federal License for chemical analyses of surface waters.

Material and methods

Studied area

Saline rivers such as the Chernavka, the Lantsug, the Khara, the Bolshaya Samoroda et al., flow into hypersaline Lake Elton (Fig. 1), located in Volgograd Region of Russian Federation (49° 13' N 46° 40' E). Climate of the area is arid; air temperature in summer is up to 41.1°C (Zinchenko & Golovatyuk 2010). Values of main physical and chemical parameters of the rivers are given in Table 1. Bottom sediments are black silt or silty sand. Water flows of the rivers are regulated by both underground water and rain water (Zinchenko & Golovatyuk 2010). Banks and mouths of the rivers are important landing sites for abundant migratory birds, for instance, little stints *Calidris minuta* Leisler. These water birds feed primarily of chironomid larvae (Kasatkina & Shubin 2012).

Reared material and its analysis; male, pupa and larva

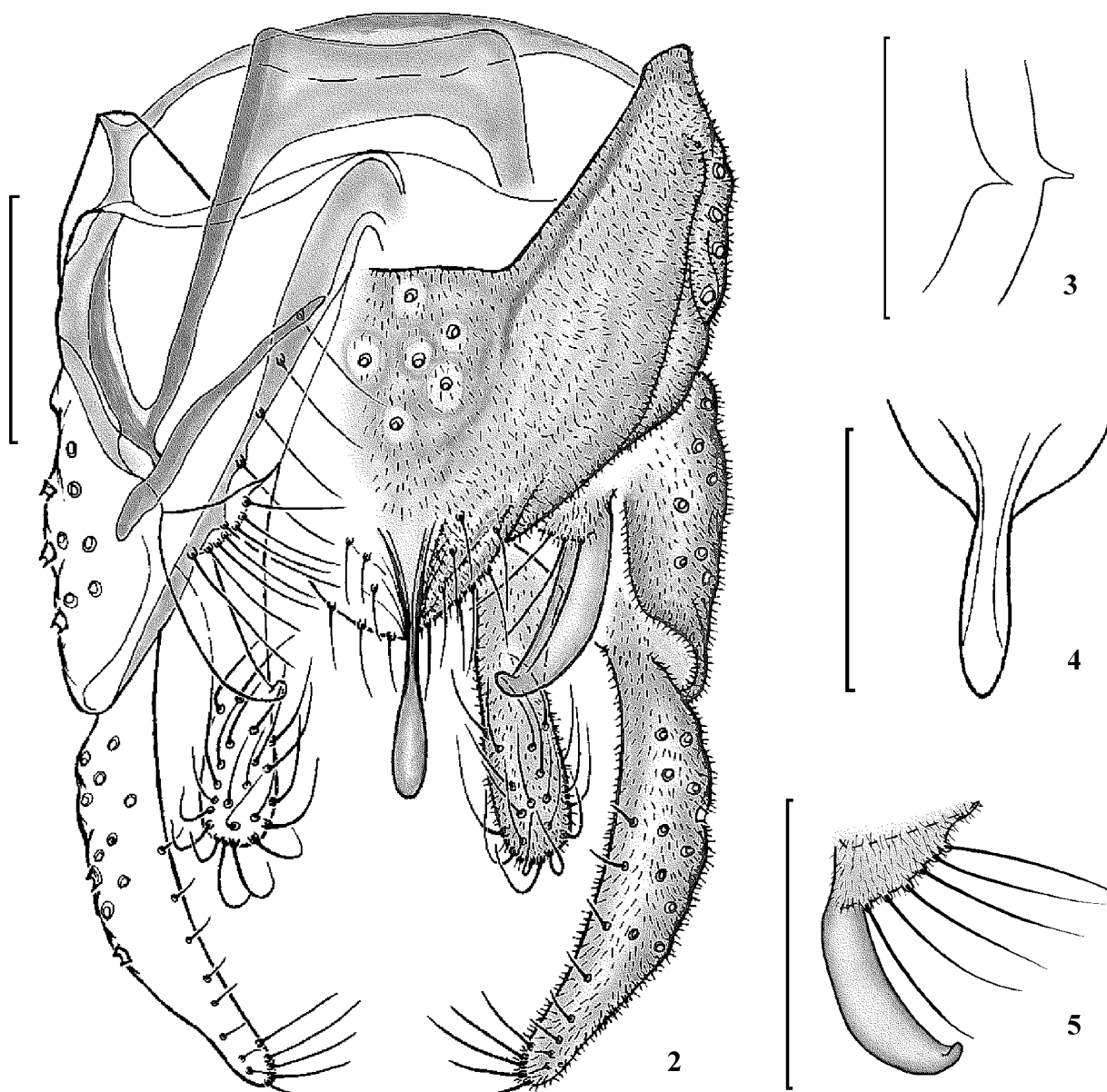
The imago males and pupae were reared under laboratory conditions (salinity 17.2 g l⁻¹; T 24°C) from larvae of *C. salinarius* from the Chernavka River.

Material was fixed in 70% ethanol and mounted in Fora-Berlese solution. Morphological terminology and abbreviations follows Strenzke (1959), Sæther (1980), Webb & Scholl (1985), Webb *et al.* (1985), Shobanov (1989, 2003, 2005). Male: TL/WL total length of male over wing length; HIR—head width to interocular distance; AR—length of apical flagellomere 13 to length flagellomeres 1–12; CP—head width to length of palpal segments 1–4; Al/Pl—length of antenna to length of palpal segments 1–4; VR—length of Cu to length of M; P₁₋₃—legs; LR—length of tarsomere 1 to length tibia; SV—length femur plus tibia to tarsomere 1; BV—length of femur, tibia and tarsomere 2–5; BR—longest seta of tarsomere 1 divided by minimum width of tarsomere 1; tiR—length of ti to length ta, fore legs; GsR—length of gonostylus to its width; HR—length of gonocoxite to length of gonostylus. Pupa: ALR—length of anal lobe to its width. Larva: AR—length of basal antennal segment to combined length of remaining segments; L1—length of basal segment of antenna; L2—length of second segment of antenna; MS—distance between tops of first lateral teeth of mentum; R—distance from ring organ to base of basal segment of antenna; ROR—length of basal segment of antenna to distance between base of basal segment and ring organ; VmPR—width ventromental plate to its length; VmPSR—mean width of the two ventromental plates to distance between ventromental plates; W1—width of basal segment of antenna.

Cytogenetic studies

For karyological study the last instar larvae of *C. salinarius* were collected in mouth length of the Lantsug River—28.IV.2007, the Chernavka River—18.VIII.2006, and the Khara River—17.VIII.2006. The karyotype and chromosomal polymorphism have been studied in 14 larvae from the Chernavka River, 52 larvae from the Lantsug River and one larva from the Khara River.

For cytogenetic analysis, the larvae were fixed in a 3:1 mixture of 96% ethanol and glacial acetic acid and kept in a refrigerator. Polytenic chromosome squashes from salivary glands were prepared using the routine aceto-orcein method (Keyl & Keyl 1959; Kiknadze *et al.* 1991). Chromosome arms A, E and F were mapped according H.-G. Keyl (1962), and arms C and D according G. Dévai *et al.* (1989), using *Chironomus piger* Strenzke, 1956 banding sequence as a standard. Arms B and G did not map because of complicated chromosome rearrangements in these arms in comparison with *C. piger*. Each banding sequence was designated by the symbol of species, the symbol of the chromosome arm, and the number of the sequence, for example, salA1, salA2, salB1 etc. The homozygous combinations of the banding sequence were designated as salA1.1, salB1.1, etc. and the heterozygous ones, as salA1.2, salG1.2. The polytenic chromosomes were photographed with the equipment of the Joint-Access Center of Microscopy Analysis of Biological Objects of the Siberian Branch of the Russian Academy of Sciences (Axioskop 2 Plus microscope, AxioCam CCD camera, and Axio Vision 4 software package; Zeiss, Germany). The photographs of chromosomes were made at the objective magnification 100x.



FIGURES 2–5. Imago male of *C. salinarius* Kieffer. 2—hypopygium, dorsal view; 3—lateral tubercles on tergite IX; 4—anal point, dorsal view; 5—superior volsella, dorsal view. Scale bar 100 μ m.

Ecological studies

For ecological study the population dynamics of *C. salinarius* (and other species; see Zinchenko *et al.* 2011) were studied by regular sampling in middle and mouth region (max. depth 120 cm) of the seven rivers (Chernavka, Solyanka, Bolshaya Samoroda, Malaya Samoroda, Khara, Lantsug, and Karantinka), flowing into a closed basin hypersaline Lake Elton (Fig. 1). Three seasonal samples were taken: often in summer (August 2006–2012; July 2011), spring (April 2007; May 2011, 2012) and autumn (September 2008), which resulted in 152 sampling occasions. Some stations were sampled once or two times (Karantinka, Malaya Samoroda), while others were sampled up to 20 times. Each sample of benthic fauna on soft substrates from each site in the centre of middle and mouth of the rivers was taken in replicate (8 x) with an Ekman grab (25 cm²) to obtain quantitative samples or by a hydrobiological scrubber, mouth width 20 cm, sweeping distance 1 m. Samples were taken in middle of rivers and or/near banks, when river width was small. Samples were fixed with 4% formaldehyde in situ transferred to plastic containers and then sorted with a sieve (300 μ m) in the laboratory. Synchronously some chemical measurements of

the surface water were done. The following parameters of the river were measured in situ: water temperature (T), dissolved oxygen (DO), pH and salinity (SI). The principal ions in total dissolved solids were taken in the rivers (Table 1). The type of sediment was described visually. We chose natural approach that would reveal the salinity tolerances and preferences of this species. Our goal is to show that *C. salinarius* responded positively to high variable salinity in the rivers. For fatty acid analyses, live stage IV chironomid larvae of dominant species were withdrawn from the benthos samples in August 2010. In the Lantsug River, *C. salinarius* and *Chironomus* (*Chironomus*) *aprilinus* Meigen, 1818 were collected. We studied the potential biochemical quality of the larvae based on the essential highly unsaturated fatty acid (HUFA) contents in their biomass. We also evaluated the fatty acid composition and contents of the dominant taxa and estimated the flux of HUFA from the studied saline rivers to land via chironomid potential emergence. A detailed description of the fatty acid analysis is given (Sushchik *et al.* 2007; Zinchenko *et al.* 2011).

The material of rearing larvae, pupae and males imago are kept in the Institute of Biology and Soil Science, Russian Academy of Science, Far East Branch, Vladivostok; karyological slides are kept in the Institute of Cytology and Genetics, Russian Academy of Science, Siberian Branch, Novosibirsk.

Results

Systematics

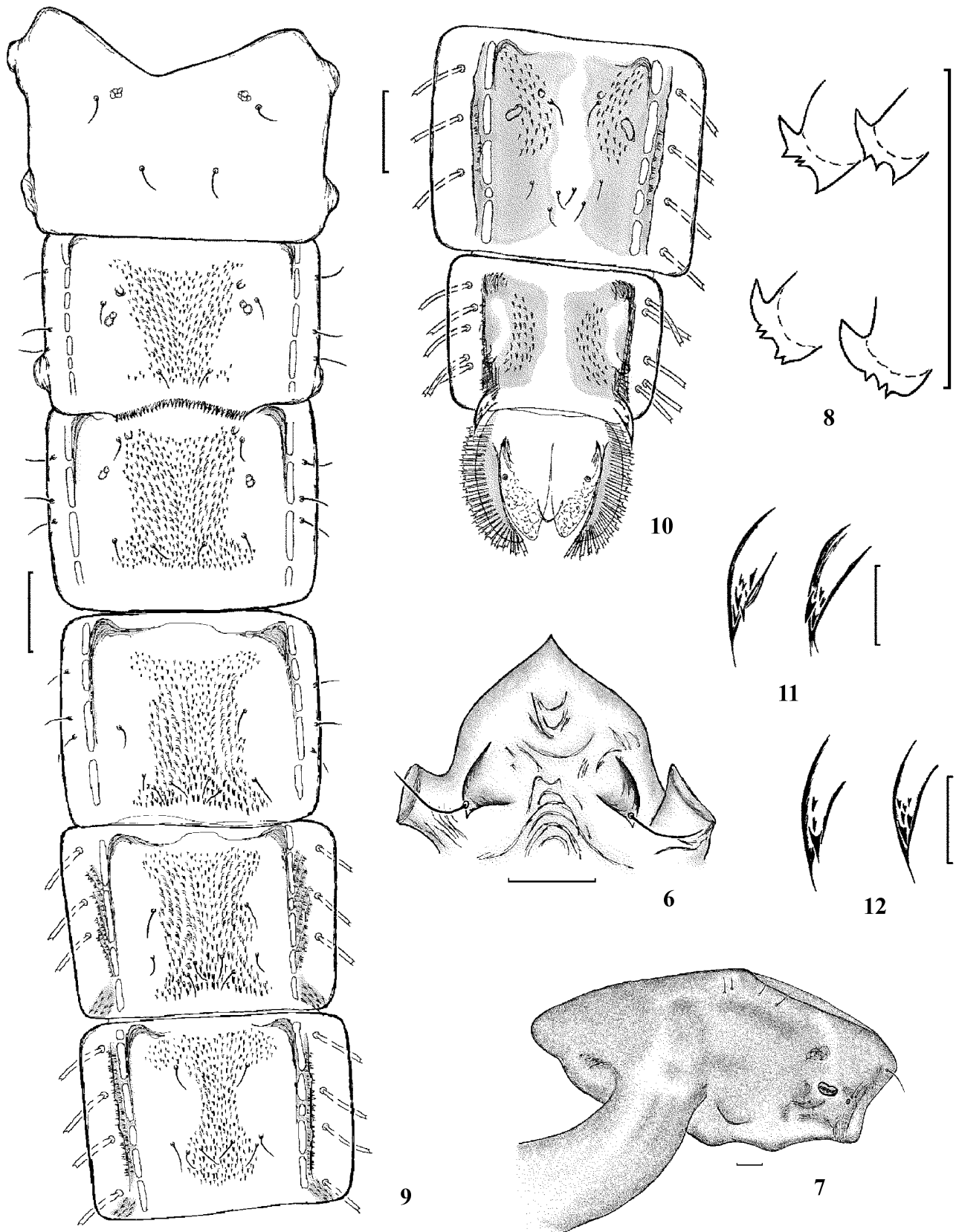
Chironomus salinarius Kieffer, 1915

(Figs 2–18)

Chironomus salinarius Kieffer in Thienemann, 1915: 451; Goetghebuer (1928: 62); Thienemann, Strenzke (1951: 15); Strenzke (1959: 22, fig. 11); Keyl & Keyl (1959: 1); Keyl (1962: 464); Linevich & Erbaeva (1971: 156, figs 73–80); Michailova (1973: 348), (1980: 141), (1989: 108); Pinder (1978: 114, fig. 145D); Sasa (1978: 20, figs 57–63); Grinchuk (1979: 44), (1984: 751); Pankratova (1983: 139); Ali & Majori (1984: 17); Webb & Scholl (1985: 353); Webb, Scholl & Ryser (1985: 373); Ceretti *et al.* (1987: 289); Chun (1989: 37); Yoon & Chun (1992: 1); Ali *et al.* (1994: 35); Drake & Arias (1995: 195); Sasa (1996: 94, fig. 8); Mirabdullayev *et al.* (2004: 101); Suemoto *et al.* (2004: 107); Fuentes *et al.* (2005: 289); Kim *et al.* (2005: 21); Ree & Yum (2006: 63, fig. 1); Gascon *et al.* (2007: 419); Ponti *et al.* (2007: 79); Marchini *et al.* (2008: 1076); Cartier *et al.* (2010: 637); Istomina *et al.* (2012: 55, figs 16–21); Kasatkina & Shubin (2012: 95).

Material. Male, pupa, larva, Russia, Volgograd region, the Lake Elton basin, the Chernavka River, 29.VI.2012, leg. L. Golovatyuk (rearing); male, pupa, larva, the same location, 6.VII.2012, leg. L. Golovatyuk (rearing); male, pupa, larva, the same location, 7.VII.2012, leg. L. Golovatyuk (rearing).

Diagnostic characters. The imago males of *C. salinarius* Kieffer can be separated from some species by following combination of features: dark brown or black colour midges, total length 5.0–6.2 mm, wing length 2.40–3.25 mm, AR 2.84–3.20, LRP₁ 1.36–1.40, BRP₁ 5.0–7.9, Ac 18–22, Dc 19–25, Pa 6–7, Scts 17–35, Sq 15–21, legs yellowish brown, except to dark brown proximal and apical ends of femur, proximal ½ and apical end of tibia, ta_{1–5} gradually darken to end, tergite IX with 6–8 median setae, anal point expanded in apical third, superior volsella E-type, gonostylus the widest at proximal third. The pupa differs following combination of features: total length 6.0–7.0 mm, segment I with anterior and posterolateral Pedes spurii B; tergite VII in proximal half part with two patches of shagreen; tergite VIII with two patches of shagreen medially; tergites V–VII laterally with longitudinal rows of spinules; tergites V–VI with posterior-lateral patches of spines. The larva can be distinguished from the other members of the *salinarius*-type larva by following features: AR 1.85–2.15, L1 68–85 µm, L2 17–22 µm, blade reached to the segment 5, ring organ situated in the proximal 2/3 basal segment; pecten epipharyngis with 17–24 teeth; anterior margin of the base maxilla with 1–2 tubercles; mandible II-type, with 1 yellowish dorsal tooth, dark brown apical and 3 inner teeth; type of mentum by the characters of the median teeth—I/II; type of mentum by the degree of development of the 4th lateral teeth—I; ventromental plate with 57–69 short striae, not reached inner spines series, type of stria termination IIIA, VmPR 0.36–0.45. VmPSR 1.6–2.4.



FIGURES 6–12. Pupa of *C. salinarius* Kieffer. 6—frontal apotome; 7—thorax; 8—median hooks in the posterior margin of tergite II, lateral view; 9—tergites I–VI; 10—tergites VII–IX; 11–12—variability of anal spurs. Scale bar 100 μ m.

Male (n=3). Total length 5.0–6.2 mm; wing length 2.4–2.5 mm. Total length / wing length 2.08–2.58.

Coloration. Thorax and abdomen dark brown or black. Legs yellowish brown, except to dark brown proximal and apical ends of femur, proximal ½ and apical end of tibia, ta_{1-5} gradually darken to end.

Head. Head width 680 µm. Height of eyes 376–520 µm. HIR 6.07. Frontal tubercles finger-shaped, length 30–36 µm, width 12 µm. Antenna 1320–1386 µm long. Ultimate flagellomere 990–1056 µm long. AR 3.0–3.2. Verticals 22–26. Clypeus with 25–31 setae. Cibarial pump 120–129 µm long, 84–90 µm wide. Maxillary palp 606–657 µm long, lengths of last 4 palpomeres (in µm): 42–60; 147–156; 168–174; 243–270. CP 1.12; AI/PI 2.00–2.18.

Thorax. Acrostichals 18–22; dorsocentrals 20–25; prealars 6–7; supraalars 1–2. Scutellum with 22–35 setae.

Wing. Length 2.4–2.5 mm, width 0.64–0.68 mm. Veins R, R_1 with 41–46 setae, R_{4+5} with 11–16 setae. Squama with 15–20 setae; brachiolum with 2–3 setae. VR 1.0–1.06.

Legs. Spurs of middle tibia 24 and 30 µm, of hind tibia 24 and 30 µm long. TiR 1.96–2.08. Lengths and proportions of legs as in Table 2.

TABLE 2. Lengths (in µm) and proportion of legs *C. salinarius*

P	fe	ti	ta_1	ta_2	ta_3	ta_4	ta_5
P_1	1078–1122	1012–1100	1430–1496	792–836	594–638	528	264–308
P_2	1166–1276	1056–1166	594–682	396–440	286–308	176–220	154
P_3	1320–1408	1320–1430	968–1056	594–616	440–462	264–286	176–198

P	LR	SV	BV	BR
P_1	1.36–1.38	1.49	1.60–1.64	5.7–7.9
P_2	0.56–0.62	3.39–3.74	2.72–2.89	3.1–5.6
P_3	0.73–0.75	2.67–2.73	2.36–2.57	2.2–5.4

Hypopygium (Figs 2–5). Tergite IX with 6 median setae. Lateral tubercles sometimes present on tergite IX. Laterosternite IX with 5–8 setae. Anal point 90–105 µm long, 12–15 µm wide, expanded in the apical 1/3, sometimes the widest at about middle (depends on the position of hypopygium). Transverse sternapodeme 93–111 µm long, without oral projections. Phallapodeme 135–144 µm long. Gonocoxite 180–186 µm long, with 5–6 inner setae. Total length of superior volsella 75–90 µm, with 6–10 basal setae; height of apical finger-shaped part 60–69 µm, width—18–21 µm, base (15–21 µm high, 30–42 µm wide) cover microtrichia (E-type). Inferior volsella 135–144 µm long, with 32–40 setae. Gonostylus 156–165 µm long, 36–39 µm wide, widest at proximal 1/3, with 1 apical seta (15 µm long) and 3–4 subapical inner setae. GsR 4.1–4.6. HR 1.11–1.15.

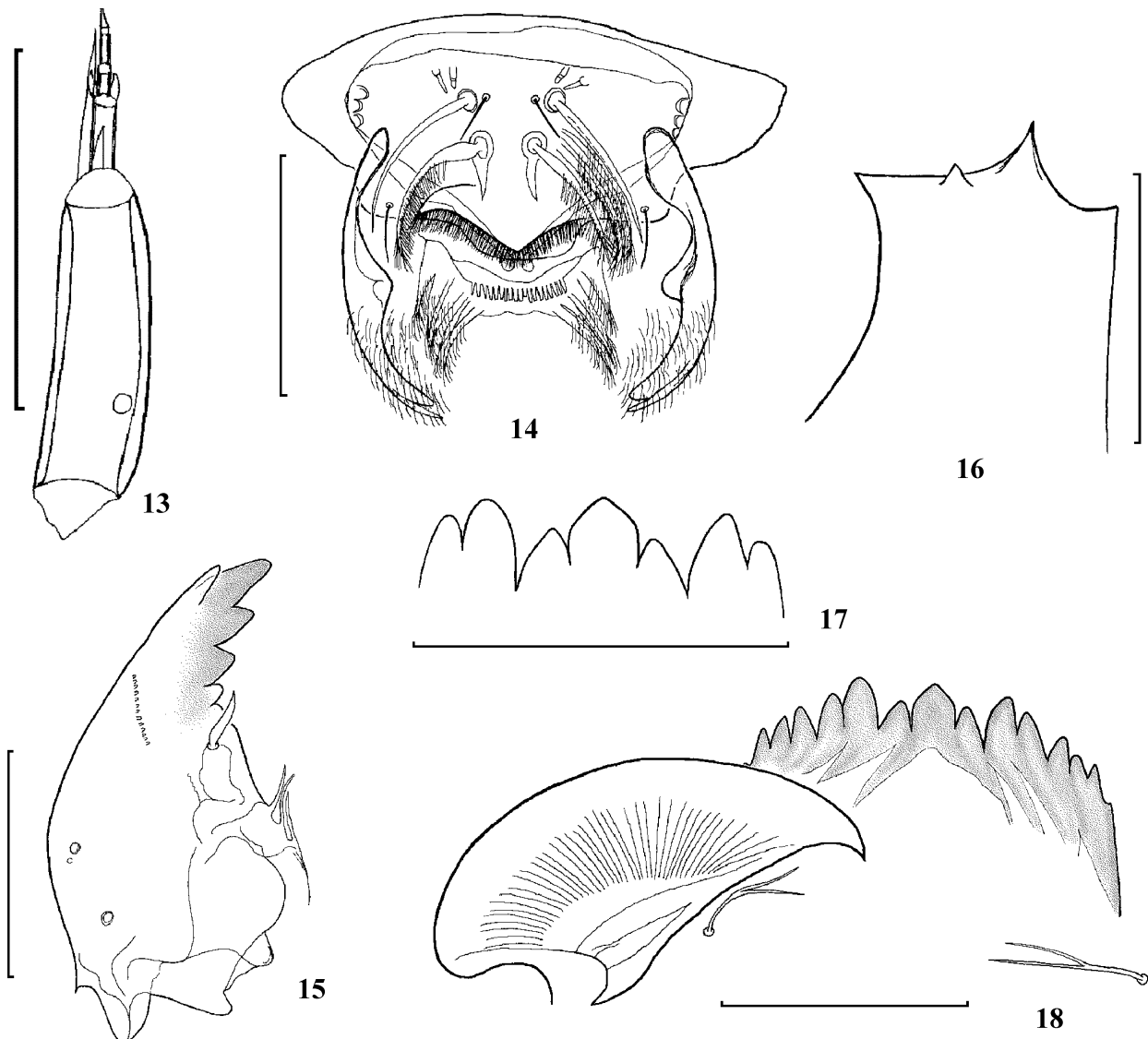
Pupa (n=3, males). Total length 6.6–7.0 mm.

Cephalothorax (Figs 6–7). Cephalic tubercles conical 63–75 µm long, 45–48 µm wide; frontal setae 66–75 µm long (Fig 6). Base of thoracic horn 78–90 long µm, 45–60 µm wide. Prealar tubercle developed. Pc_1 51–54 µm long, Pc_2 51–54 µm long; MAs 111–135 µm long; Dc_1 45–75 µm long, Dc_2 45–90 µm long, Dc_3 45–51 µm long, Dc_4 48–60 µm long; distance between setae Dc_1 – Dc_2 10–84 µm, Dc_3 – Dc_4 18–36 µm, Dc_2 – Dc_3 123–156 µm. Wing sheath length 237–246 µm, width 60 µm (Fig 7).

Abdomen (Figs 8–12) length 5.0–5.5 mm. Tergites II–VI covered by square shagreen; tergite VII in proximal half part with two patches of shagreen; tergite VIII with two patches of shagreen medially. Tergites V–VII laterally with longitudinal rows of spinules; tergites V–VI with posterior-lateral patches of spines. Anal segment without shagreen, but in posterior part granulose. Hook row with 64–78 hooks; tops of median hooks with 1–5 little teeth (Fig 8). Conjunctives IV/V and V/VI with little spinules, sometimes on conjunctives V–VI spinules absent. Sternite I usually with several spinules laterally; sternite II medially with shagreen and lateral longitudinal rows of spinules; sternites III in proximal part with median path of shagreen and several spinules laterally; sternite IV usually with several spinules laterally; sternites V–VI with posterior-lateral group of spinules; sternites VI–VIII with anterior-lateral group of shagreen. Segment I with anterior and posterolateral pedes spurii B (Fig 9). Segment VIII with single dark brown spur, sometimes spur with three tips, covered by 5–7 dorsal spines (Figs 11–12). Segment II–IV

each with 3 L setae, V–VI each with 4 LS setae, VII with 3–4 LS setae, VIII with 5 LS setae. Anal lobe with 65–78 lamelliform setae. Length of anal lobe 400–432 μm , width 416–464 μm . ALR 0.86–1.04.

Fourth instar larva (n=3).



FIGURES 13–18. Larva of *C. salinarius* Kieffer. 13—antenna; 14—labro-epipharyngeal region; 15—mandible; 16—anterior margin of base maxilla; 17—median and 1–2 lateral teeth; 18—mentum and ventromental plate. Scale bar 100 μm .

Head (Figs 13–18) yellowish brown, length 0.50–0.55 mm, width 0.45 mm, cephalic index (W/L) 0.82–0.90. Frontal apotome 408–440 μm long, 136–176 μm wide. Distance between setae S_1 – S_1 69–84 μm , S_2 – S_2 96–114 μm , S_3 – S_3 93–123 μm , S_4 – S_4 96–129 μm , S_5 – S_5 96–129 μm . Antenna 111–123 μm (Fig. 13), length of each segment (in μm): 69–84; 18; 6; 9; 6. AR 1.85–2.15. Maximal width of basal segment 24–27 μm . Ring organ distribute in the proximal 2/3 basal segment; distance from ring organ to base of antenna 24 μm ; ROR 2.9–3.5. Blade 42 μm long, reached to the segment 5; accessory blade 12 μm . S I 45–48 μm long, S II 54–60 μm long, S III 21 μm long, S IV 6 μm long. Pecten epipharyngis 42–45 μm long, with 17–24 teeth. Premandible 102–105 μm long, with 2 teeth; premandibular seta simple 15–24 μm long (Fig. 14). Mandible 159–180 μm long, 87–99 μm wide; with 1 yellowish dorsal tooth, dark brown apical and 3 inner teeth; length apical tooth 21–24 μm (Fig. 15). Type mandible II (some degree of pigmentation present on 3rd tooth and tooth partly free on lower margin). Seta subdentalis 15–21 μm long. Pecten mandible with 12–14 setae. Mola with 1–3 long spines (15–21 μm). Mandible basally with 8–16 radial grooves. Maxillary palp 24–30 μm long, 15–21 μm wide. Anterior margin of the base maxilla with 1–2 tubercles (Fig. 16). Mentum 141–153 μm wide, central tooth conical 12–16.5 μm , median trifold tooth 27–42 μm wide, distance between first lateral teeth 48–66 μm , distance between second lateral teeth 72–84 μm ; distance

between the top of first lateral teeth 42–54 μm (Figs 17). Type of mentum by the characters of the median teeth—I/II; type of mentum by the degree of development of the 4th lateral teeth—I. Ventromental plate 150–168 μm wide and 60–78 μm height (Fig. 18). Distance between ventromental plates 30–48 μm . Ventromental plates with 60–65 striae. VmPR 0.36–0.45. VmPSR 1.6–2.4.

Body. Lateral and ventral tubules absent. Procercus 21 μm height and 12 μm wide.

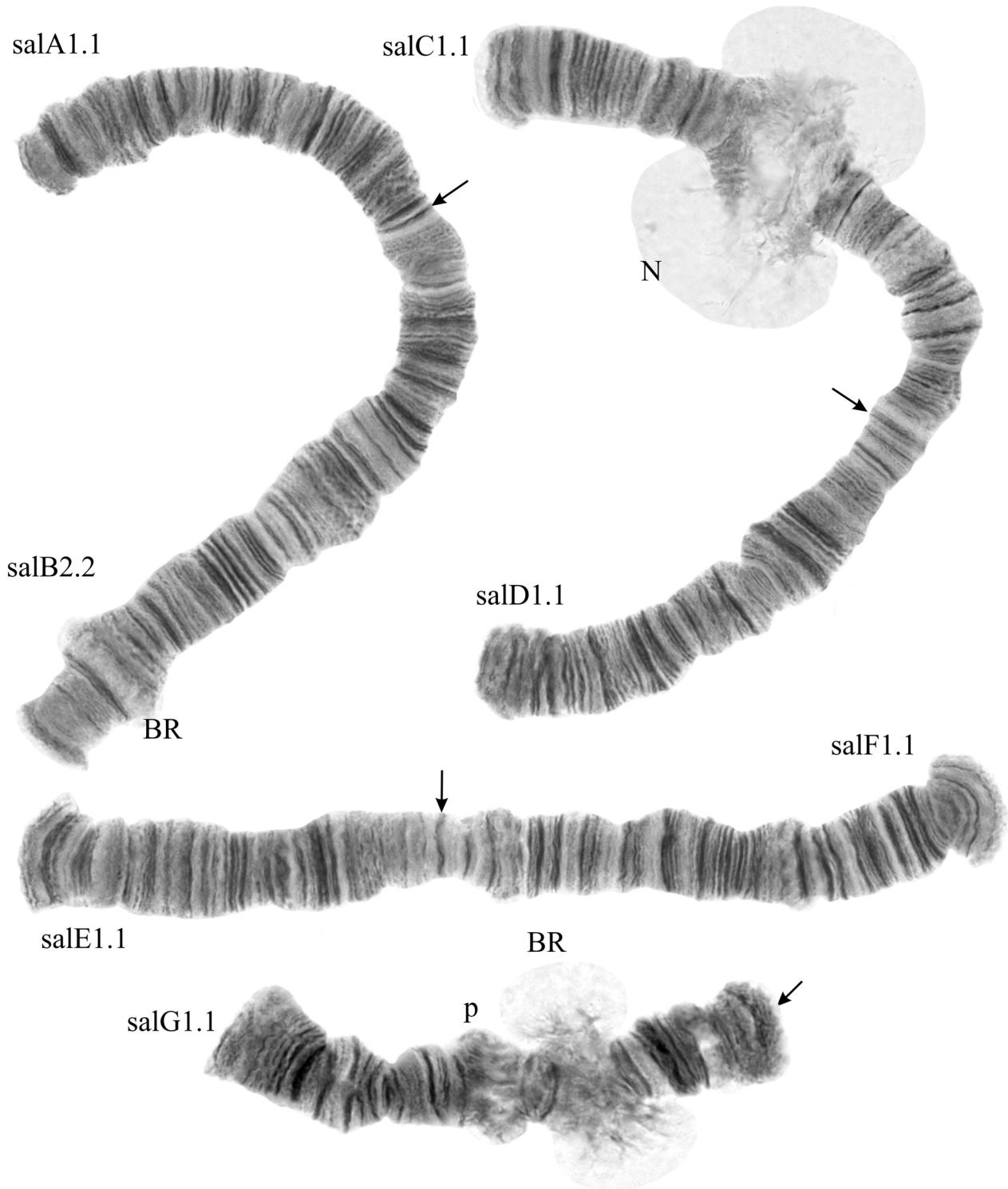


FIGURE 19. Karyotype of *C. salinarius* Keiffer. Karyotype: salA1.1, salB2.2, salC1.1 etc. note the symbols of genotypic combinations of banding sequences in chromosome arms; N—nucleolus, BR—Balbiani ring, p — puff. Arrows indicate centromeric band.

Karyotype

The karyotype of *C. salinarius* (Fig 19) has the haploid number of chromosomes $n = 4$, with the chromosome arms combination: AB CD EF G (cytocomplex “thummi”). Chromosomes AB and CD are metacentric, EF is submetacentric, and G is telocentric. The centromeric bands are not heterochromatinized and it is difficult to identify them. The regular nucleolus (N) is single and located in the middle of arm C, which is the good mark of the *C. salinarius* karyotype (Fig.19). Balbiani rings (BR) are developed in arms G (1–2), and B (1). Chromosome polymorphism was revealed in arms A, B, E and G.

Banding sequences in *Chironomus salinarius*

Arm A (Figs 20, 26, 27) has three banding sequences: salA1, salA2 and salA3. Sequence salA2 differs from salA1 by simple lengthy pericentric inversion (Figs 20, 26); salA3 differs from dominant salA1 by long complex inversion (Fig. 27).

salA1 1a–2e 12c–9a 15e–13a 4a–6c 16a–17h 18a–19f 8g–6d 2f–3i C

salA2 not mapped

salA3 not mapped

Arm B (Figs 21, 28) is recorded in two banding sequences salB2 and salB3. The banding sequence salB1 which was represented in the first description (Keyl & Keyl 1959) was not found in populations studied. The salB2 dominates in populations studied, while salB3 is found only several times. The salB2 differs from the salB1 by spread simple inversion and meets as homozygote or heterozygote with a rare banding sequences salB3. The salB3 differs from the salB2 by simple short inversion and meets only as heterozygote. The banding sequences in arm B of *C. salinarius* have not been mapped because of a series of complex inversions in comparison with pigB1.

Arm C (Fig. 22) is monomorphic with the sequence salC1:

salC1 1a–d 6g–17c (6c–f?) 1e–6b 18a–22g C

The large nucleolus is situated in the middle of arm.

Arm D (Fig. 23) is monomorphic with the single banding sequence salD1:

salD1 1ab 10a–15b 19h–18a 8c–9e 4a–8b 1c–3g 17f–15c 20a–24g

Arm E (Figs 24, 29) is polymorphic and has two sequences salE1 and salE3. The banding sequence salE2, which was found in German population (Keyl & Keyl 1959) is absent in populations from the Lantsug and the Chernavka Rivers. The salE3 was found only once as a heterozygote and may be refer to the rare sequences.

salE1 1a–d 3b–e 10b–5a 4hg 3a–1e 4f–a 3f 10c–13g C

salE3 not mapped

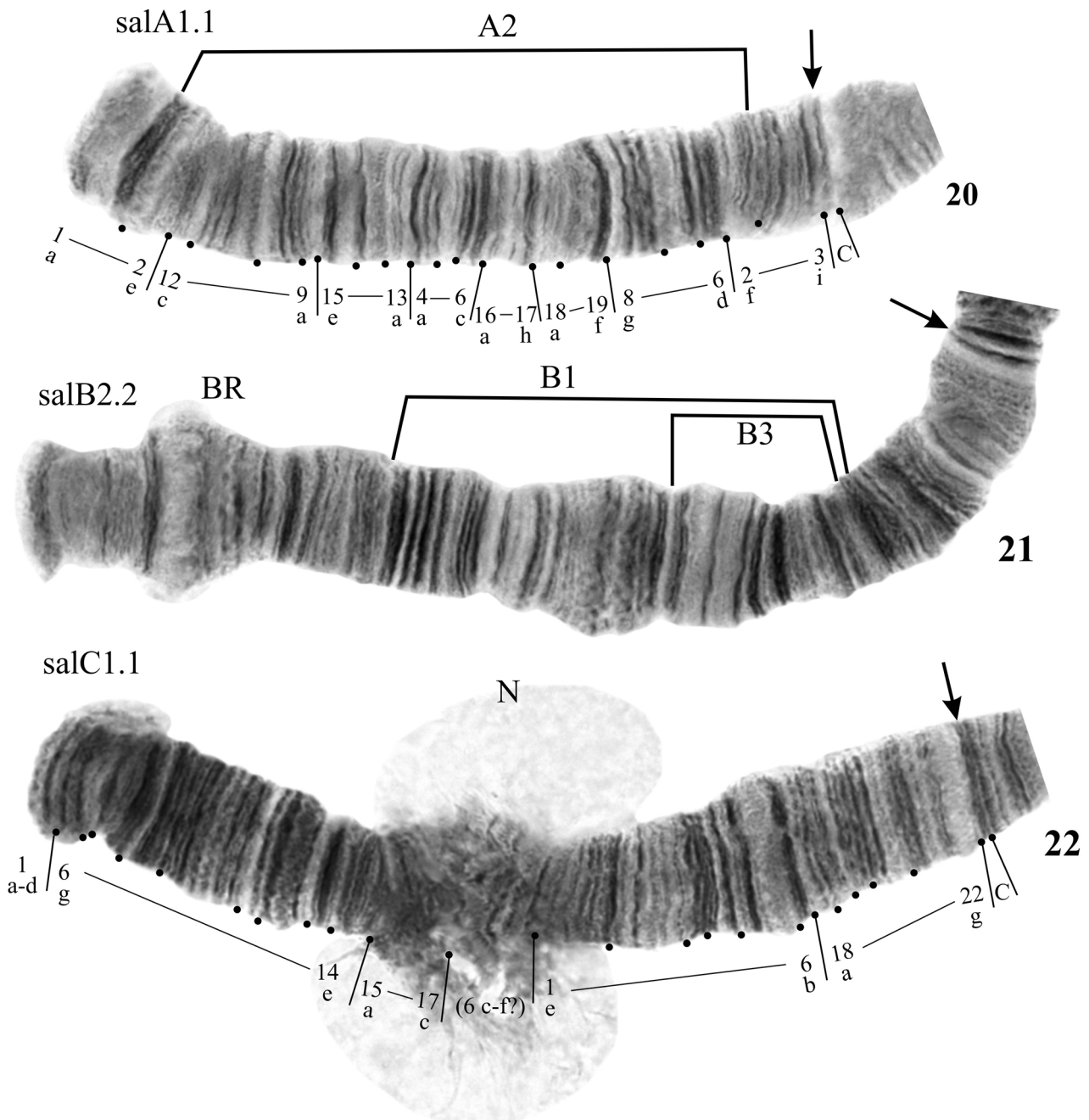
Arm F (Fig. 25) is monomorphic with banding sequence salF1:

sal F1 1a–2a 14e–2b 16a–17d 14f–15i 18a–23f

Arm G (Figs 30–33) is not mapped. It has two banding sequences salG1 and salG2. The salG2 differs from salG1 by inversion of a small region located near centromere and was found as a rule in heterozygous state (Fig. 33), but it was observed only once as homozygote in population from the Lantsug River (Fig. 31). The peculiarity of arm G in *C. salinarius* is an appearance of extra Balbiani ring lying in region between the basic Balbiani ring (BR1) and centromere. This peculiarity was recorded by Keyl & Keyl (1959) in some larvae (Fig. 32). Besides Balbiani rings in arm G puff developed in different degree is situated in immediate proximity to BR1 (Figs 30, 33).

TABLE 3. Cytogenetical characteristics of chromosomal polymorphism in the natural populations of *C. salinarius*

Cytogenetical characteristics	Populations	
	Lantsug River n=52	Chernavka River n=14
Heterozygote individuals in population, %	0,48	0,57
Mean number of heterozygote inversions per individual	0,48	0,71
Number of banding sequences	10	11
Number of genotypic combinations of banding sequences	11	11

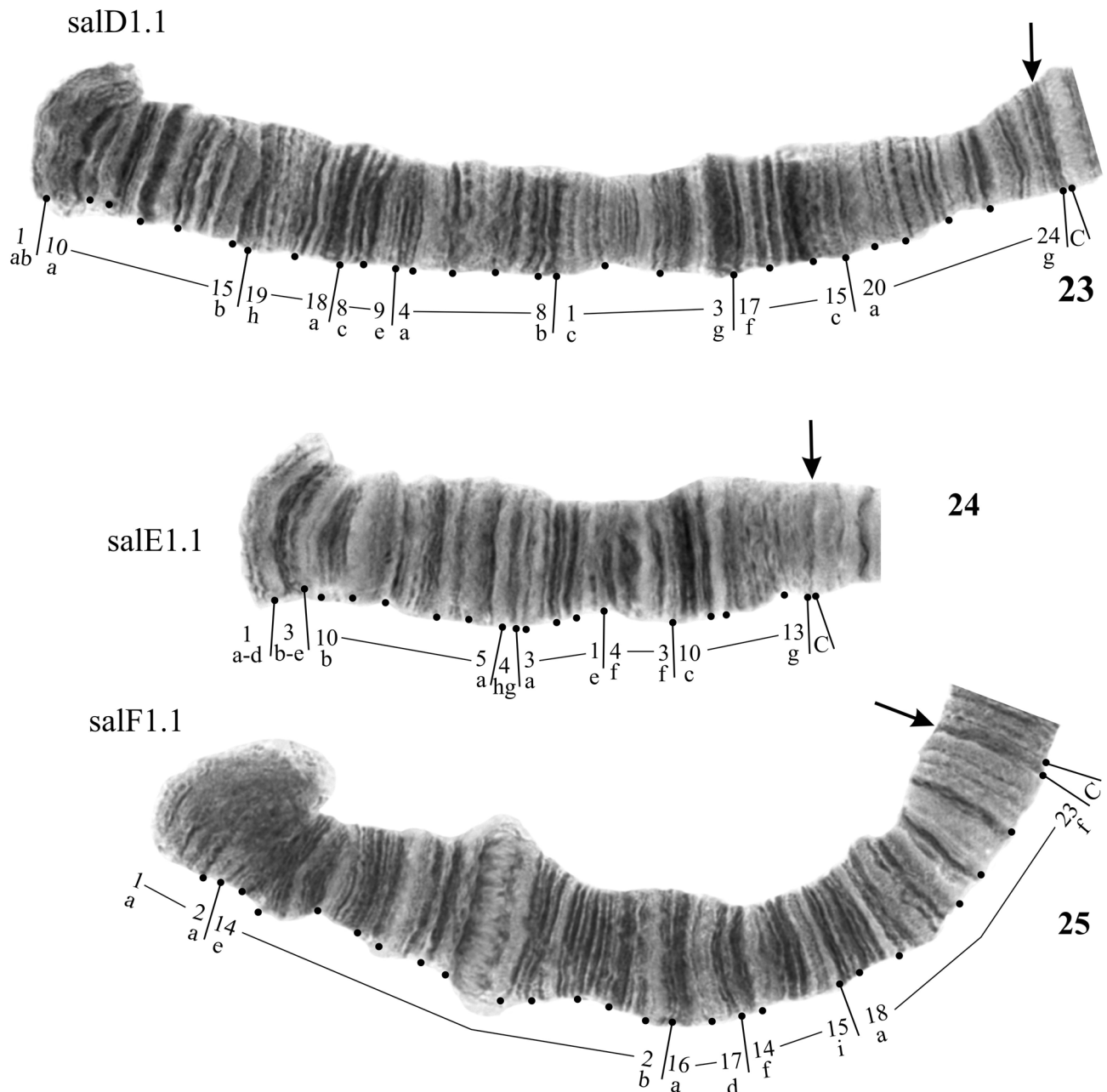


FIGURES 20–22. Homozygotes in *C. salinarius* Kieffer karyotype: 20—salA1.1; 21—salB2.2; 22—salC1.1. Brackets above and below chromosome arms indicate the localization of inversions. Here and in all other figures numbers and small letters under chromosome arms correspond to banding sequences. The rest designations are the same as in Fig. 19.

Chromosomal polymorphism

Chironomus salinarius is a polymorphic species, so chromosomal rearrangements were found in the half of the larvae studied. In total, 16 banding sequences have been known now in *C. salinarius* with 12 of these observed in the populations studied and 4 of them were found in Bulgaria, Germany and Ukraine and not found in Russia. The average number of heterozygous inversions per larva varied from 0.48 (the Lantsug River) to 0.71 (the Chernavka River) (Table 3). Four from seven chromosomal arms were polymorphic, three banding sequences were found in arm A, two banding sequences were found in each of arms B, E, and G. It should be noted that the frequency of inversions occurrence in different arms is unequal. The main contribution in the populations polymorphism put a short arm G, nearly a half of larvae studied had a heterozygote salG1.2, whereas frequencies of inversions

occurrence in A, B, and E arms were low (0.019–0.077). Ten banding sequences were found in population of the Lantsug River, and 11 banding sequences in the Chernavka River, which formed 11 genotypic combinations. It is important to underline that the karyotype of *C. salinarius* is the most reliable feature for species identification.

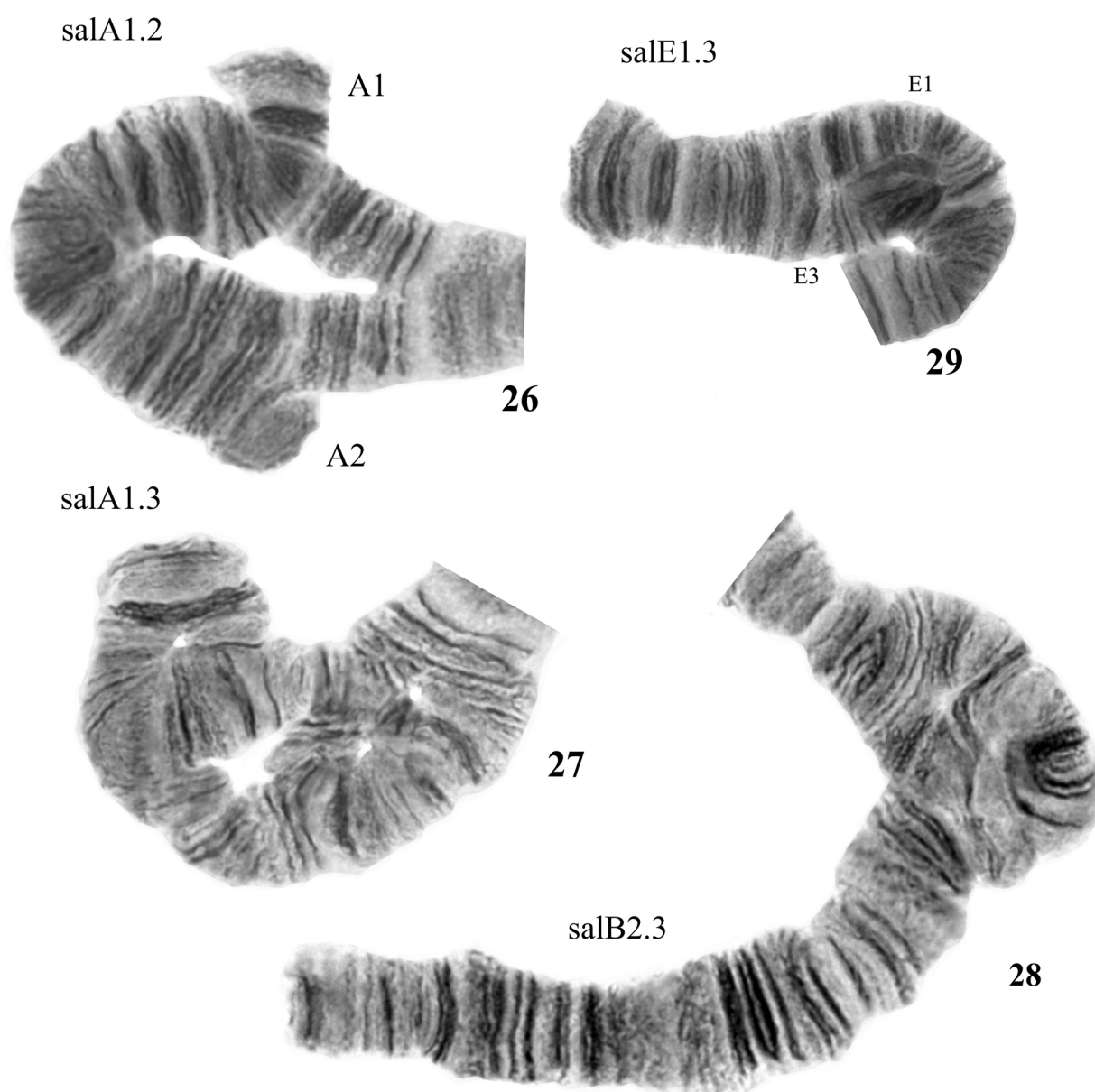


FIGURES 23–25. Homozygotes in *C. salinarius* Kieffer karyotype: 23—salD1.1; 24—salE1.1; 25—salF1.1. The designations are the same as in Fig 19.

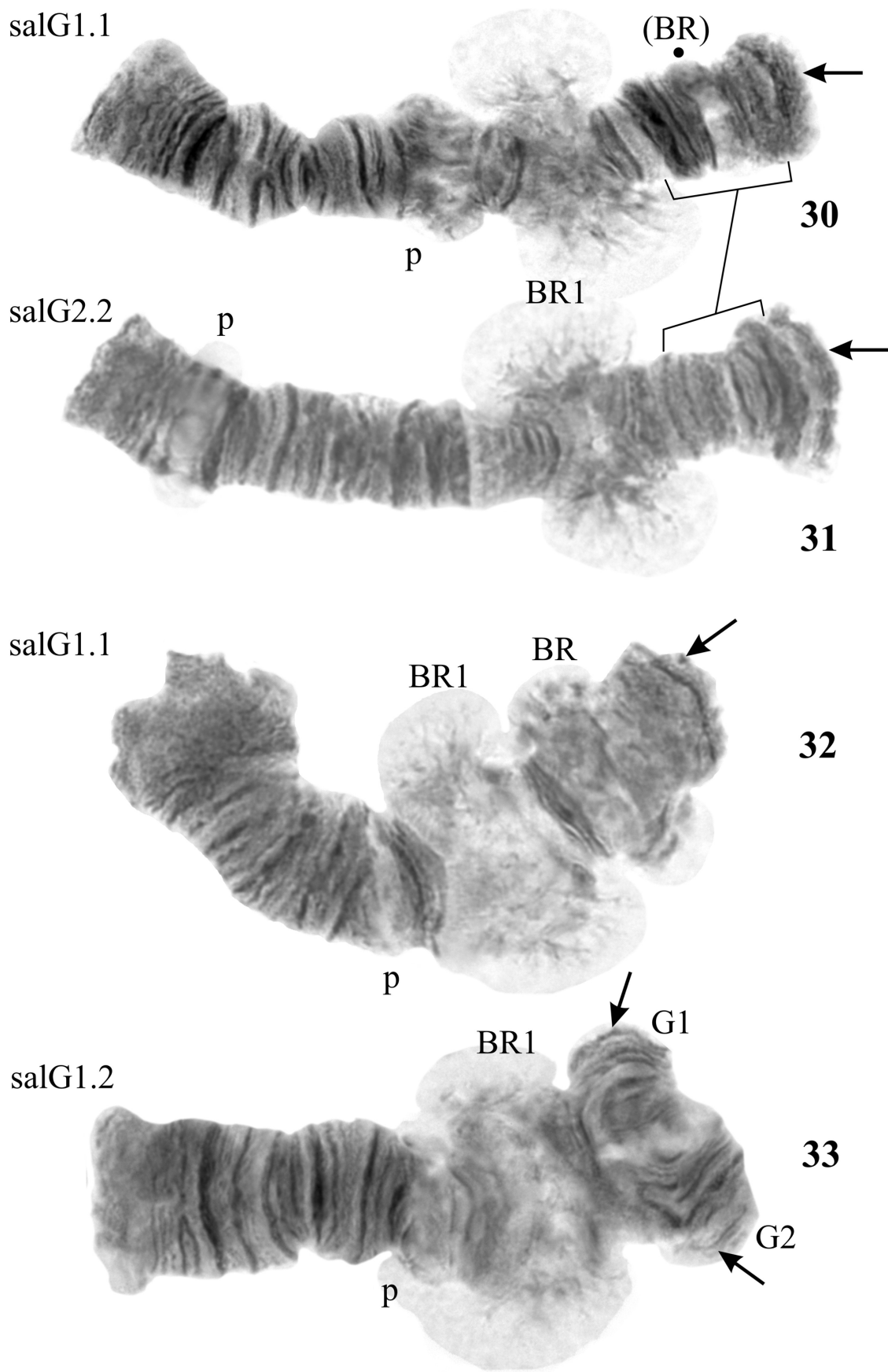
Ecology

Chronomus salinarius is a common species in the Chara, the Lantsug, the Chernavka, the Solyanka, the Bolshaya Samoroda, the Malaya Samoroda and the Karantinka Rivers (Fig. 1) from the Lake Elton basin. The larvae inhabit the black and grey sandy mud on depths of 0.05–1.1 m, at the velocity of 0.02–0.4 m s⁻¹ (Table 1). They do not occur between dense vegetation. The larvae live in the various saline rivers, T—12–32°C and concentration of dissolved oxygen of 4.3–44.4 mg l⁻¹ (Table 1). They probably need an oxygen percentage of at least 40 %. The larvae of *C. salinarius* inhabit waters with the pH from 7.2 to 10.0. Several behavioural adaptations enable *C.*

salinarius larvae to live in the saline rivers on salinity 9.1 g l^{-1} – 41.1 g l^{-1} . The total average biomass of chironomids was significantly higher than that of all other taxa in the saline rivers. In terms of abundance the *C. salinarius* larvae represented 11.4% of the total chironomid's fauna collected over the six years in the saline rivers (2007–2012). In the mouth regions of saline rivers flowing into Lake Elton in sites with silt-sand bottoms they occurs in different biocenoses together with *Cricotopus* (*Cricotopus*) *salinophilus* Zinchenko, Makarchenko & Makarchenko, 2009, *Chironomus aprilius* Meigen, 1918, *Microchironomus deribae* (Freeman, 1957), *Glyptotendipes salinus* Michailova, 1987. Frequency (% of samples) in the Chernavka River and the Lantsug River is 33–50%. The abundance of 61280 ind. m^{-2} represented in the Chernavka River (12.V.2012) appeared to be a maximum population value for larvae of this species. In other rivers the contribution of *C. salinarius* ranged from $< 2\%$ in the Karantinka River and the Bolshaya Samoroda River. The larvae of *C. salinarius* which had different density (ind. m^{-2} , $\text{mean}\pm\text{SD}$) at diapason of salinity over the studied period in each river are given in Fig. 34. Density of *C. salinarius* accounted for 49–66% of total abundance of zoobenthos from salinity to 13 – 31.6 g l^{-1} in the Chernavka and the Lantsug Rivers.



FIGURES 26–29. Heterozygotes in *C. salinarius* Kieffer karyotype: 26—salA1.2; 27—salA1.3; 28—salB2.3; 29—salE1.3. The designations are the same as in Fig 19.



FIGURES 30–33. Homozygotes and heterozygotes in arm G *C. salinarius* Kieffer: 30, 32—salG1.1; 31—salG2.2; 33—salG1.2. A black point and (BR) in Fig 30 designate a region from which extra BR is developed.

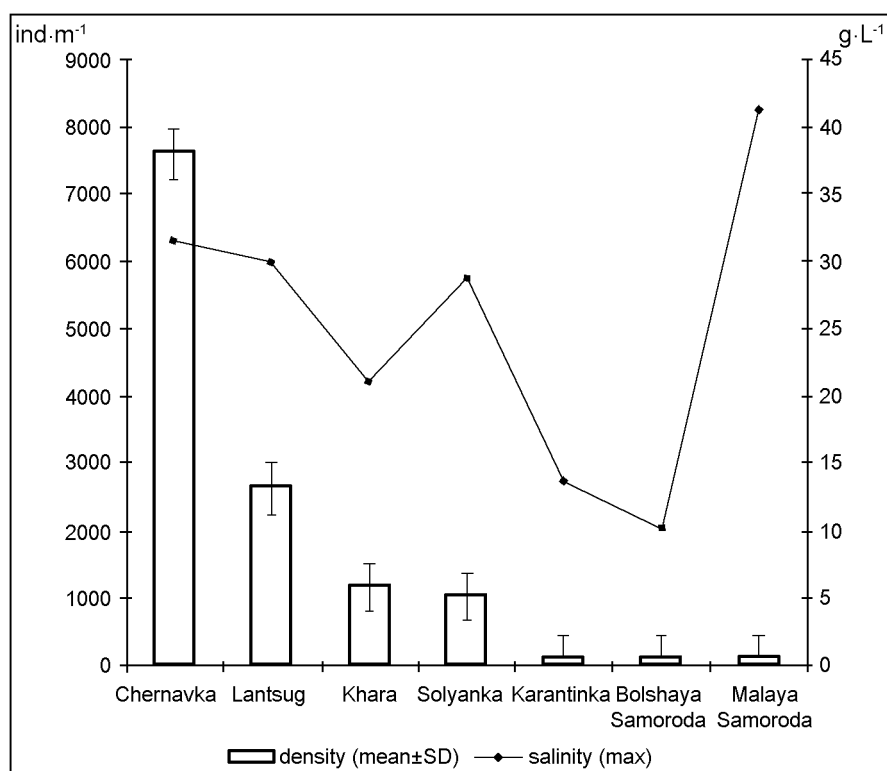


FIGURE 34. Density (ind m⁻² mean±SD) larvae of *C. salinarius* Kieffer and salinity (max) at places of habitats larvae during the studied period (August 2007–2012, July 2011), (April 2007, May 2011, 2012), (September 2008).

The population studied was multivoltine with a probable average of three or four generations per year, with overlapping cohorts and a predominance of third-fourth instars larvae in samples. There are no data on feeding of larvae of *C. salinarius* in the available literature. The make use of fatty acid analysis was found differences between fatty acid composition of the chironomid larvae of *C. salinarius* and the bottom sediments indicated a selective feeding of larvae. The larvae had significantly higher levels of odd-number fatty acid 15:0 synthesized by bacteria (Desvillettes *et al.* 1997; Napolitano 1999) compared to the level of this acid in the sediments (Zinchenko *et al.* 2014). This finding is in a good agreement with data on selective feeding of larvae of *Chironomus* (*Chironomus*) *plumosus* (Linnaeus, 1758) in a freshwater reservoir (Gladyshev *et al.* 1999).

Distribution

Chironomus salinarius is widely distributed in Palaearctic (Sæther & Spies 2004). This species is widespread in the Far East, namely, in Korea (Chun 1989; Ree & Yum 2006), in Japan (Sasa 1978), and China (Wang 2000). In Russia *C. salinarius* recorded from Irkutsk region (Linevich & Erbaeva 1971), Volgograd region (Istomina *et al.* 2012), Kabardino-Balkarskaya Republic (Karmokov 2013) and Primorye Territory (Makarchenko *et al.* 2005).

Discussion

Our data on morphology of imago male, pupa and larva are compared with previous ones. Our description of imago males is almost indistinguishable from the ones by other authors (Table 4). Pupae from our material are characterized by the presence on tergite VII of two groups of spinules in the proximal half and of lateral longitudinal strips of shagreen, while pupae described Linevich & Erbaeva (1971) have tergite VII with anteromedial field of shagreen and posterolateral groups of spinules. In our material, the larvae have higher index

values L1/W1, L1/L2 and L1/MS, but at the same time, the distance between the tops of the first lateral teeth (MS) and the width of the ventromental plates is slightly smaller in European specimens (Table 5). Thus, larvae and pupae described from various lakes and rivers can be distinguished morphologically this is possibly due to different habitat conditions.

At the present time 15 species of *Chironomus* with larvae of the salinarius-type are described in Holarctic Region (Andersen 1937; Pedersen 1978; Webb 1969; Butler 1982; Wülker & Butler 1983; Kiknadze *et al.* 1992; Michailova 1992; Hirvenoja & Michailova 1998; Shobanov *et al.* 2002). According to Lindeberg & Wiederholm (1979) *C. salinarius* very close to *Chironomus* (*Chironomus*) *anchialicus* Michailova, 1974 and *Chironomus* (*Chironomus*) *valkanovi* Michailova, 1974 with larvae also living in brackish water. Morphological features of male, pupa and larva of this species cannot be compared with the ones of *C. salinarius* due to insufficient information (Michailova 1974). The imago males of other species of *Chironomus* with larvae of the salinarius-type living in freshwater are characterized by dark brown or black color of body, large body, more or less parallel-side anal point and superior volsella of the E-type, as observed in *C. salinarius*. The values of AR, LR_p and BR_p of *C. salinarius* overlap with other species (Table 6). Pupae are known for thirteen species of the 15 species of *Chironomus* with larvae of the salinarius-type. Here again a comparative morphological analysis is hindered by lack of information. The larvae of *C. salinarius* differ from all the other known species of salinarius-type for the smaller length of antennal segments (L1 68–85 µm, L2 17–22 µm) (Table 7).

The conclusion is that it is necessary to use karyological method for a correct identification of *Chironomus* species within the salinarius-type, insignificant differences being present in imago male, pupal and larval morphology.

The karyotype and cytophotomaps of polytene chromosomes of *C. salinarius* which are the most accurate taxonomic feature on larva stage are submitted. The karyotype structure and chromosomal polymorphism of *C. salinarius* were studied in two populations from the rivers of the Lake Elton basin. When compared the chromosomal polymorphism from rivers the Lantsug and the Chernavka populations to Germany (Keyl & Keyl 1959) and Black Sea populations of Bulgaria and Ukraine (Michailova 1973, 1980, 1989; Grinchuk 1979, 1984) differences in a range and frequency of inversion sequences were recorded. Populations studied from Russia differed from German and Bulgarian populations by homozygous inversion in arm B: homozygote salB1.1 have been met in West European populations, whereas homozygote salB2.2 was fixed in the Chernavka and the Lantsug populations and both homozygote salB2.2 and heterozygous salB1.2 have been found in Odessa populations (Keyl & Keyl 1959; Michailova 1978; Grinchuk 1979, 1984). Arm G is polymorphic in populations studied in comparison with populations from Germany and Bulgaria. The polymorphism level in arm G is high and brings the main contribution to the polymorphism of populations. Heterozygotes salG1.2 with small frequency (0.9–5.4%) was met in Odessa populations (Grinchuk 1984). Arm A is polymorphic in populations studied as in Bulgarian populations, but the frequency of salA1.2 considerably lower, then in Bulgarian one. Arm D is monomorphic in populations studied and presented by salD1.1, whereas in Bulgarian populations this arm is polymorphic and submitted by heterozygote salD1.2, but in Black sea limans salD1.1 and salD2.2 are presented. In the Lantsug and the Chernavka populations arm F is monomorphic with banding sequence salF1, whereas in Odessa limans about half of larvae were heterozygous. In total the *C. salinarius* karyotype includes 16 banding sequences (Keyl & Keyl 1959; Michailova 1980, 1989; Grinchuk 1984, present communication). The populations of *C. salinarius* from the Lake Elton basin differs cytogenetically from the populations of Germany and Black Sea populations of Bulgaria and Ukraine that is evidence of adaptability of chromosome polymorphism.

Chironomus salinarius was reported in many environments with widely-varying levels of salinity. In a shallow coastal lagoon in Spain, *C. salinarius* was present in water whose salinity varies from 6 to 60 g l⁻¹ (Drake & Arias 1995), in the Emporda Wetlands (NE Iberian Peninsula), Gascón *et al.* (2007) this species was described as a mesohaline to metahaline species (salinity varying from 7 to 43 g l⁻¹). *Chironomus salinarius* was also reported in the western basin of the Aral sea (Mirabdullayev *et al.* 2004), and in a hypersaline environment (salinity over 65 g l⁻¹) in the Mediterranean area, with a range of salinity varying from 14 to 45 g l⁻¹ (Marchini *et al.* 2008; Ponti *et al.* 2007; Ali & Majori 1984; Ali *et al.* 1994; Ceretti *et al.* 1987; Fuentes *et al.* 2005; Suemoto *et al.* 2004).

TABLE 4. Morphological characteristics of males of *C. salinarius* from various authors

Morphological characters	Literature data				Our data	
	Strenzke 1959	Linevich & Erbaeva 1971	Sasa 1978, 1996	Ree & Yum 2006	Volgograd region	Primorye Territory*
TL	—	5.5–6.0 mm	—	—	5.0–6.2 mm	6.0 mm
WL	3.1–3.8	4.0 mm	4.5; 3.78–4.12 mm	3.3 mm	2.4–2.5 mm	3.25 mm
AR	3.3–4.4	4.0	3.4; 3.65–4.02	3.5	3.0–3.2	2.84
LRP ₁	—	1.5	1.3; 1.30–1.36	1.4	1.36–1.38	1.40
BRP ₁	5.4–6.3	—	4.6; 3.7–4.2	—	5.7–7.9	5.0
Frontal tubercles L/W	—	—	32/12 µm	27/11 µm	30–36/12 µm	42–56/14 µm
CL	—	—	18–24	26	25–31	18
Ac	17–33	—	21–33	—	18–22	20
Dc	19–33	—	25–42	17–18	20–25	19–24
Pa	4–9	—	5–8	—	6–7	6
Scts	29–40	—	26–37	25	22–35	17
Sq	—	—	18–40	—	15–20	21
Color of legs	dark brown, for example to yellowish median part of femur	—	dark brown, except to yellowish median spot on femur P ₁ and P ₂	yellowish brown, with darker proximal and apical ends of femur, proximal ½ and apical end tibia, ta _{1–5} dark brown	yellowish brown, except to dark brown proximal and apical ends of femur, proximal ½ and apical end of tibia, ta _{1–5} gradually darken to end	same color
Anal point	expanded in the apical third	parallel-sided	expanded apically	slightly expanded distally	expanded in the apical third	expanded in the apical third
Median setae on tergite IX	1–6	1–4	12	13	6	8
Gonostylus	abruptly narrow in apical third	without apical constriction, gonostylus widest at about middle	abruptly narrow in apical third	abruptly narrow in apical third	not abruptly narrow in apical third	abruptly narrow in apical third

Note: * According to Makarchenko *et al.* 2005.

TABLE 5. Morphological characteristics of larvae of *C. salinarius* from different authors.

Morphological characters	Literature data (by Webb & Scholl 1985; Webb, Scholl & Ryser 1985)	Our data
L1	68–85 µm	69–84 µm
L2	17–22 µm	18 µm
W1	24–31 µm	24–27 µm
R	–	24 µm
L1/W1	2.4–2.8	2.9–3.1
L1/L2	3.5–4.5	4.0–4.7
L2/W1	0.6–0.8	0.7–0.8
R/L1	0.23–0.40	0.29–0.35
MS	56–75 µm	42–54 µm
L1/MS	1.1–1.4	1.5–1.6
L2/MS	0.25–0.35	0.33–0.38
W1/MS	0.40–0.56	0.50–0.57
Type of mentum	I, rarely II	I
Type of median trifid tooth	I/II	I/II
Type of mandible	III, sometimes II	II
Number of epipharyngeal teeth	13–25	17–24
Size of ventromental plates	260–310 µm	150–168 µm
Number of ventromental plate striae	57–69	60–65
Number of ventromental plate outer hoors	12–19	–
Size of ventromental plate inner spines	5–7	–
Outer hoors not reaching plate margin	+	+
Striae short, not reaching inner spine series	+	+
Anterior part of stria ridge longitudinally undivided	+	+

High productivity seems to be a characteristic of all saline waters, chironomid larvae in two saline rivers (including *C. salinarius*) had high monthly production in August, 16.7 g m⁻² month⁻¹, dry weight, which was nearly comparable to annual production in some freshwater rivers (Zinchenko *et al.* 2014). In addition, for instance, in a brackish pond in coastal lagoon system of northern Adriatic Sea (Italy) annual production of *C. salinarius* was up to 69.2 g of ash-free dry weight per m² per year (Ponti *et al.* 2007). Abundance of *C. salinarius* larvae, reported in available literature was up to ~44 000 ind. m⁻² (Cartier *et al.* 2010). Besides, larvae of *C. salinarius* (together with *C. aprilinus*) are dominant taxa in brackish wetlands, which are a very important habitat for water birds (Fuentes *et al.* 2005). Moreover, migratory birds selectively consumed larvae of *C. salinarius* among other potential prey, which were abundant and readily available (Sanchez *et al.* 2006). The larvae represented up to ~97% of the total number of captured prey of water birds (Estrella & Masero 2010). Really, the larvae, pupae and adults of *C. salinarius* are the stable food of many water birds in ecosystem of the Lake Elton basin (Kasatkina & Shubin 2012). *Chironomus salinarus* from the saline rivers were found to be selective feeders, preferring bacteria (Zinchenko *et al.* 2014). Food selection is probably an adaptation to meet the energy requirements where extreme abiotic conditions are observed. As a last point it is emphasized that huge emergence of *C. salinarius* from water bodies near settlements caused a serious nuisance problem for humans in several countries (Ree & Yum 2006; Cartier *et al.* 2010). More data about ecological traits geographical distribution, limits of tolerance and diet peculiarities, are recommended for environmental management.

TABLE 6. Measurements and ratios of males of the species of *Chironomus* with larvae of the salinarius-type.

Species	Morphological characteristics			
	WL	AR	LRp ₁	BRp ₁
<i>C. albimaculatus</i> Shobanov et al., 2002	—	≈6.5	—	—
<i>C. brevidentatus</i> Hirvenoja, Michailova, 1998	4.3–4.5	3.7–4.0	1.36–1.39	2.1–2.5
<i>C. cucini</i> Webb, 1969	4.1–5.2	3.7	1.35	“beard very short”
<i>C. hyperboreus</i> Staeger, 1945	5.2	≈5.0	1.24–1.34	“long beard”
<i>C. islandicus</i> Kieffer, 1913	3.7–5.5	4.5–5.5	1.06–1.17	5.1–6.3
<i>C. neocorax</i> Wülker, Butler, 1983	3.93–4.8	3.7–4.0	1.43–1.48	2.2–2.8
<i>C. prior</i> Butler, 1982	4.04–4.80	4.89–5.48	1.10–1.20	4–7
<i>C. salinarius</i> Kieffer, 1915	2.4–4.12	2.84–4.4	1.3–1.5	5.7–7.9
<i>C. tardus</i> Butler, 1982	4.23–5.36	5.09–5.78	1.01–1.14	4–7
<i>C. trabicola</i> Shobanov et al., 2002	—	5.8–7.3	1.03–1.08	5.25–6.78
<i>C. tuvanicus</i> Kiknadze et al., 1992	3.3–3.5	3.9–4.2	1.2–1.4	3.0–3.7

TABLE 7. Measurements of antenna of the species of *Chironomus* with larvae of the salinarius-type (length in μm)

Species	Morphological characteristics				
	L1	L2	W1	L1/L2	L1/W1
<i>C. albimaculatus</i> Shobanov et al., 2002	131–167	—	—	—	—
<i>C. cucini</i> Webb, 1969	134.5–154.2	26.3–39.5	37.6–39.5	3.42–5.14	2.67–4.10
<i>C. hyperboreus</i> Staeger, 1945	86.5–114.7	33.8–37.6	33.8–37.6	2.42–3.22	2.55–3.22
<i>C. islandicus</i> Kieffer, 1913	124.1–148.5	26.3–33.8	43.2–52.6	3.88–5.0	2.36–3.29
<i>C. major</i> Wülker, Butler, 1983	116.6–150.4	30.1–37.6	45.1–67.7	3.50–4.33	2.11–3.07
<i>C. neocorax</i> Wülker, Butler, 1983	92.1–105.3	26.3–30.1	37.6–45.1	3.25–4.0	2.33–2.45
<i>C. salinarius</i> Kieffer, 1915	68–85	17–22	24–31	3.5–4.7	2.4–3.1
<i>C. tardus</i> Butler, 1982	79.8–114.7	30.1–32.0	45.1–56.4	3.25–3.81	1.92–2.45
<i>C. trabicola</i> Shobanov et al., 2002	152–179	—	—	—	—
<i>C. tuvanicus</i> Kiknadze et al., 1992	116–132	24–32	48–56	4.3	2.3
<i>Chironomus</i> sp. 2n=6, Lokka Michailova, 1992	118.9–127.1	32.8–41.0	32.8–36.9	3.0–3.75	3.2–3.8

Acknowledgments

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