SPIXIANA	Supplement 14	129–137	München, 15. Juli 1988	ISSN 0177–7424

Morpho-karyological description of Euryhapsis subviridis (Siebert) from the South of the Soviet Far East

(Diptera, Chironomidae)

By E. A. Makarchenko, J. J. Kiknadze and J. E. Kerkis

Introduction

The species *Euryhapsis subviridis* (Siebert) belongs to the primitive Orthocladiinae including also chironomids of the genera *Eurycnemus* Wulp, *Xylotopus* Oliver, *Brillia* K. and *Irisobrillia* Oliver (Oliver, 1981, 1982, 1985; Oliver & Roussel, 1983). Out of the genera given, only species *Brillia* and mainly *B. longifurca* K.[1] and *B. modesta* Mg. (Pankratova, 1970; Shilova, 1976) are widespread over the USSR. In addition to representatives of this genus *(B. flavifrons* Joh., *B. modesta, B. laculata* Oliver & Roussel[2], we have found two species of *Euryhapsis* Oliver: *E. cilium* Oliver and *E. subviridis* (Siebert) in the Soviet Far East, the metamorphosis of the latter being examined from larva to imago and the larvae fixed for karyoanalysis.

Chironomids of the genus *Euryhapsis* which is new to the USSR fauna at all stages of development are close to *Brillia,* however they can be easily recognized by the structure of male hypopygium (wide transverse sternapodeme, distomedial lobe of gonostylus with 2 terminal and few subterminal lamellate macrosetae and shorter than subapical lobe, by structures of pupa sternites (sternites VII–VIII each with a posterior row of spines on low protuberance) and by antenna structure of larva (the 2nd segment is never divided into 2 parts).

There are detailed descriptions of the preimaginal stages in *E. cilium* and imago of North America and Mongolia (Oliver, 1981) whereas *E. subviridis* was identified by the only male from Austria (Siebert, 1979). That is why the present work deals with description of larva and pupa of this species as well as the re-description of a male obtained in the Soviet Far East. As the larvae of *Euryhapsis* are poorly distiguished by their external morphological characters we considered it necessary to carry out the karyological analysis of *E. subviridis* and to give the first description of its karyotype demonstrating the photomap of the polytene chromosomes as well as to present information about the chromosome polymorphism in the Sakhalin population studied.

Karyotype analysis has contributed much to our current knowledge of the taxonomy and evolution on *Chironomidae* (Keyl, 1961, 1962; Martin et al., 1974; Martin, 1979; Wülker & Butler, 1983; Belyanina et al., 1983). The karyotypes of more than 130 species of the subfamily Chironominae have been most extensively studied. Based on karyological data, the taxonomic position of many species was made more precise, a number of new ones described (among which of particular interest are the sibling-species), and the phylogenetic relation of the species to each other were established (Keyl,

[1] In our opinion *B. longifurca* Kieffer, 1921 should be considered as a synonym of *B. flavifrons* Johannsen, 1905 because the male hypopygium of these species are identical.

[2] Previously this species was known only from North America (Oliver & Roussel, 1983).

1962; Martin, 1979; Wülker & Butler, 1983; Dévai et al., 1983; Ryser et al., 1983; Kiknadze & Kerkis, 1984, 1986). Other subfamilies, however, in particular Diamesinae and Orthocladiinae, have been less studied on karyological grounds. At present the number of Orthocladiinae species studied is just 40 or so, Diamesinae – about 7 (Kuberskaya, 1974, 1979, 1984; Mechelke, 1953; Michailova, 1976, 1980, 1985; Petrova, 1983), and this is clearly insufficient for a general consideration of the karyosystematics of the entire Chironomidae family.

Materials and methods

Material for morphological studies of *E. subviridis*: 2♂♂, Primorye, Khasan Region, Barabashevka River, 17.VII.1975 (E. Makarchenko); 1♂, Primorye, Dalnegorsky Region, Inza River, 1.X.1983 (E. Makarchenko); 1♂, ibid., Partizansk Region, Partizanskaya River, 18.V.1984 (E. Makarchenko); 1♂, Sakhalin Island, Tym River, 18.IX.1979 (E. Makarchenko); 20♂♂, 13♀♀, 8 pupae, 12 larvae, ibid., Sokol Village, 18.VII.1986 (E. Makarchenko). Larvae and pupae were fixed by 70% ethanol and imagines by Udemans solution.

Terminology and abbreviation to A. I. Shilova (1976) and Saether (1980).

The larvae for karyological analysis were fixed in 3:1 ethanol-acetic acid and stored in a refrigirator. Analysis was performed on squashed spreads of salivary gland polytene chromosomes prepared by the standard aceto-orcein technique. The gonads and the imaginal discs stained with aceto-orcein were used for the meiotic and mitotic metaphase chromosome spreads. Determination of larvae age was based on the morphology of the imaginal discs according to Wülker & Götz (1968). The total number of larvae studied was 28.

Euryhapsis subviridis (Siebert) comb. nov.

Siebert, 1979: 167–168 *(Brillia)*

Male (n = 8).

Total color greenish-white or yellowish white. Head brownish or reddish, eyes black, antenna brownish, thorax yellowish or greenish. Legs white or greenish, spurs of tibia black. Length, 4.5–5.9 mm; TL/WL 1.4–1.7.

Head. Verticals, 13–25; postorbitals, 6–11; clypeals, 11–28. AR 1.58–2.27. Length of last four maxillary palp segments (μ): 55–75.8; 250–345; 160–244; 157.5–247.5.

Thorax. Antepronotum with 3–6 dorsomedial and 8–16 ventrolateral antepronotals. Dorsocentrals, 36–55; prealars, 11–21; supraalars, 1; scutellars, 34–36.

Wing with micro-and macrotrichias. Length, 2.7–3.8 mm. Squamal setae, 9–51.

Legs. LR_1 0.86–0.88; LR_2 0.55–0.59; LR_3 0.57–0.75; SV_1 2.0–2.1; SV_2 3.5–3.74; SV_3 2.49–2.98; BV_1 2.17–2.38; BV_2 2.8–3.48; BV_3 2.61–2.72. Hind tibial comb consisting of 8–9 spines.

Hypopygium (fig. 1 A–H). Tergite IX with 12–28 setae; sternite IX laterally with 4–9 setae. Gonocoxite parallel-sided, without stout setae on dorsomedial margin. Superior volsella with 18–30 setae (about 30 μ of length). Distomedial lobe of gonostylus about three-quartes as long as subapical lobe and with 2 terminal and 6–8 subterminal lamellate macrosetae. Transverse sternapodeme square to rectangular with a truncated apex.

Pupa (n = 6)

Colour grey or dark grey, anal lobe yellowish or greyish; length, 6.3–8 mm. Exuviae grey.

Thoracic horn brownish-yellow or yellow; large, pointed and covered with spinules only in the upper three quarters (fig. 2A). Length of thoracic horn 481.4–572.7 μ; ratio of length of thoracic horn to width of thoracic horn 5.2–6.9.

Tergites II–V each with rough shagreen on all surface (fig. 3D); tergite VI medial with tender shagreen only. Tergites II–VI each with posterior row or rows of spines (fig. 3C, E). Tergites VII–VIII

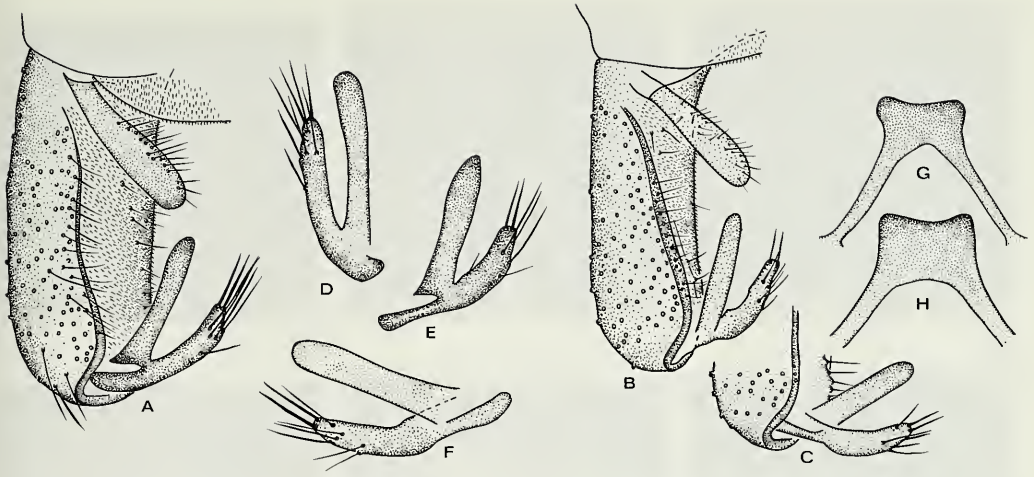

Fig. 1. Male of *Euryhapsis subviridis:* A–C – hypopygium (A – from Sakhalin Island, B–C – from Primorye), D–F – gonostylus, G–H – transverse sternapodeme (G – from Primorye, H – from Sakhalin Island).

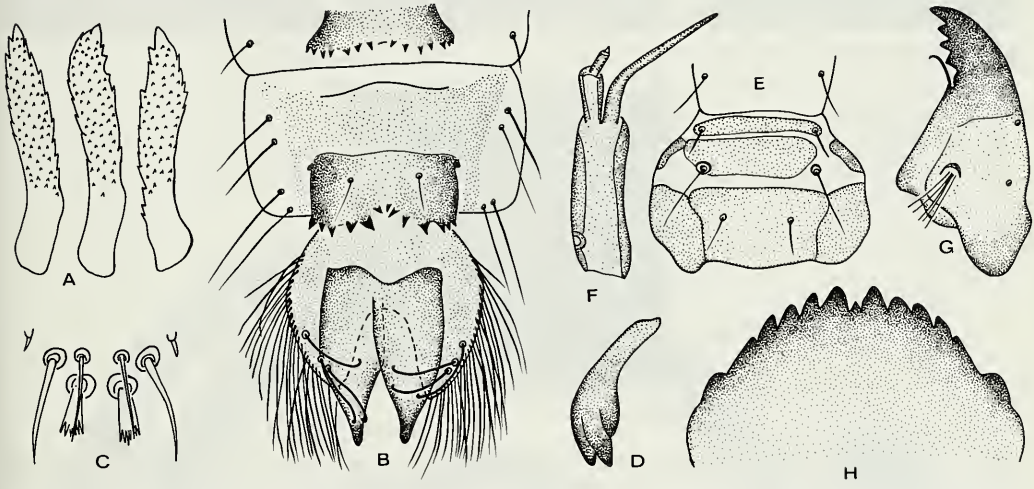

Fig. 2. Pupa (A–B) and larva (C–H) of *Euryhapsis subviridis:* A – thoracic horn; B – segments VII–IX of male, ventral; C – SI–SIV setae of labrum; D – premandible; E – frontal apotome, clypeus, and labral sclerites; E – antenna, G – mandible, H – mentum.

without shagreen and posterior rows of spines. Sternites II–III each with very tender anterior shagreen only. Male sternites VI–VIII each with a posterior row of spines, on low protuberance on VII (fig. 3F) and two low protuberance on VIII (fig. 2B); female sternites VI–VII similar to male, and two large, flap-like protuberance on VIII (fig. 3G). Number of spines of posterior row of sternites VI–VIII: 14–17; 13–14; 10–16. Conjuctives II/III–IV/V with spinules (fig. 3A–B). Segments II–VIII each with 4 L-setae; length of lateral setae of segments I–V 105.6–132 μ, of segments VI–VIII 161.7–264 . Anal lobe with complete lateral fringe of 26–34 setae and 3 anal macrosetae (fig. 2B).

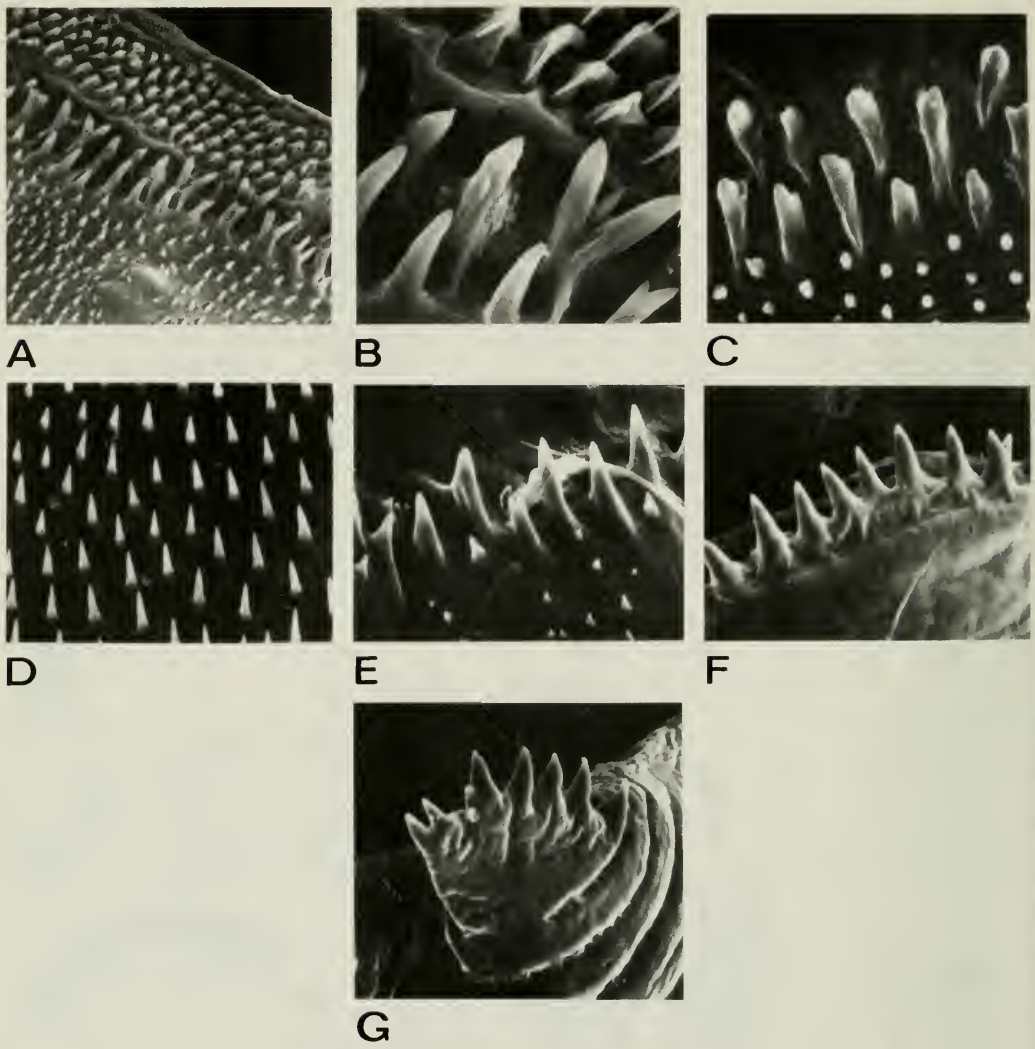

Fig. 3. Structures of tergites and sternites of pupa *Euryhapsis subviridis:* A – posterior row of spines tergite II and conjuctives II/III, ×275; B – ibid, ×525; C – posterior rows of spines tergite III, ×525; D – shagreen of tergite III, ×525; E – posterior row of spines tergite VI, ×525; F – posterior row of spines sternite VII, ×275; G – posterior row of spines sternite VIII of female, ×275.

Larva, fourth instar (n = 6)

Colour white or greenish; length, 8.2–8.9 mm. Head capsule yellow, width of head 0.4–0.5 mm; eye spot, apical half of mandible and mentum black.

Labrum granular on lateral; labral sclerites as on fig. 2E. S I setae of labrum distal plumose, S II–SIII simple, S IV present, but very small (fig. 2C).

Antenna yellowish-brown, 4-segmented; ring organ near base of first segment; antennal blade about 1.5–1.6 times as long as combined length of flagellar segments (fig. 2F); AR 1.63–1.95. Premandible distal with 3 teeth (fig. 2D).

Mandible with 5 teeth; basal tooth blunt and shorter as other inner teeth. Seta subdentalis long, apically slightly curved, ending at level of apex of second inner tooth; seta interna with 5 plumose branches (fig. 2G).

Mentum with one median tooth and 6 pairs of lateral teeth present; sixth lateral tooth lower as other lateral teeth (fig. 2H).

Procercus about 1.5–1.6 times as long as wide, posterior part strongly sclerotized; bearing 8 apical and 2 subapical anal setae, the lowest of them about 2–3 times as long as the upper one.

Oecology

Larvae inhabit the low course of foothill rivers on gravel-pebble grounds with sandy filling at the rate of 0.5–0.7 m/s.

Karyological analysis of larva of fourth instar

Observation of the diplotene nuclei and mitotic metaphases (of imaginal disc mitoses) in *E. subviridis* show three pairs of very small chromosomes (fig. 4a). Because of the somatic pairing characteristic of Diptera, the homologues lie close to each other, and the number of chromosomes discernible at the mitotic metaphase often appears to be three. Judging by the morphology of the metaphase chromosomes, two pairs are metacentrics, and the third one is submetacentric. As expected from the mitotic arrangement, in the salivary gland nuclei there are three chromosomes, each of them exhibits two paired homologues (fig. 4b). The chromosomes have been numbered I to III in order of decreasing length and tentatively given left and right ends. The major division has been numbered consecutively throughout each chromosome, and each major one subdivided into minor ones denoted by letters (fig. 5). *E. subviridis* polytene chromosomes have distinctive banding patterns making them advantageous for karyological analysis. However, we encountered difficulties when attemping to identify the centromeres because there were no associated large blocks of heterochromatin. We succeeded in defining the

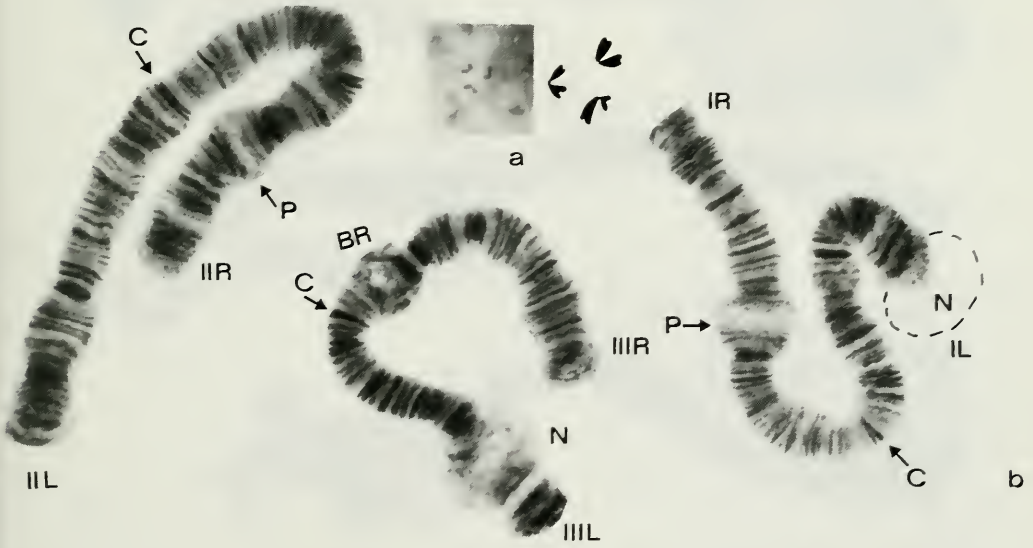

Fig. 4. The entire chromosome complement of *Euryhapsis subviridis*. a – mitotic chromosomes; b – salivary gland polytene chromosomes. IL–IR; IIL–IIR; IIIL–IIIR – chromosome arms. The arrows indicate the putative candidates for the centromeric bands. N – Nucleolar organizer, BR – Balbiani ring.

centromeric regions only after thoroughly examining of many preparations. The bands we thought to represent the centromeric regions in all the larvae studied were distinguished from all other bands of the polytene chromosome by their homogenous staining.

Chromosome I (175± 5.2 μ) is the longest of the complement. It is a metacentric chromosome containing section 1–21. The centromeric region is identified in section $^{9}/_{10}$. A characteristic feature is the presence of a nucleolus at the end of arm IL in section 1. Reviable markers of arm IL are the constrictions in sections 3 and 8 as well as dark bands in sections 4, 6 and 7. Such markers in arm IR are the constriction in section 10, heavy bands in sections 16, 17 and 18. A prominent puff develops in some larvae in section 15. Heterozygous inversions in arm IL were detected only in one larvae of all those examined (fig. 6a).

Chromosome II (168 ±8 μ) is nearly as long as chromosome I. It is a metacentric like chromosome I (fig. 5). It consists of 21 sections. The centromeric region shows two dark bands in section $^{10}/_{11}$. Arm IIL is distinguished by the presence of constriction in sections 2 and 4, clear-cut banding in section 5 and pale bands in section 8; arm IIR has a constrictions in section 11, bands distinct in sections 12, 13 and 15). There is a small puff in section 20 in all the larvae studied, and also in sections 10 and 18 in some (fig. 4–5). No nucleoli and Balbiani rings were identified in chromosome II, neither were rearrangements.

Chromosome III (152± 4.5 μ) is the shortest of the complement, and it is divided into 17 sections. It is a submetacentric (fig. 4–5). The centromere was deduced by the presence of a dark band in section 10. There is a Balbiani ring near section 9 of arm IIIR and a nucleolus in section 15. The main markers

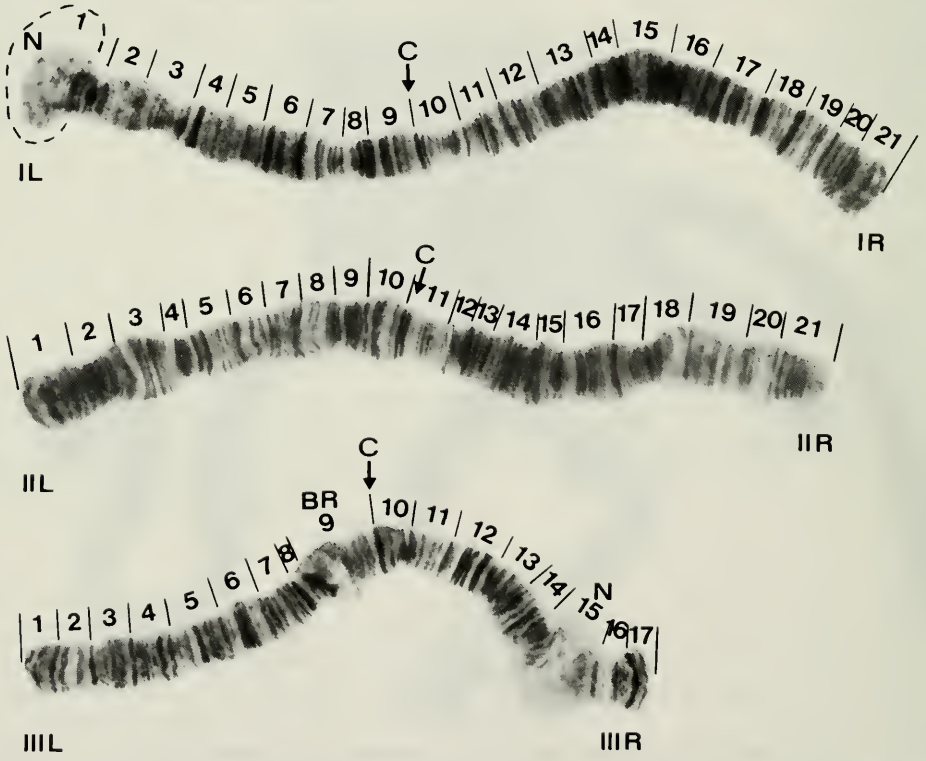

Fig. 5. A standard map of the salivary gland chromosomes of *Euryhapsis subviridis*. The designations are the same as in fig. 4.

of arm IIIL are groups of bands in sections 4, 5, 6, 7 and 8; and those of arm IIIR are groups of pale bands in section 11 and dark ones in sections 12 and 17 (fig. 5). Heterozygous inversions were observed in both arms in 33 % of the larvae (fig. 6).

The distinctive morphological features of the salivary gland of *E. subviridis* deserve some comments. The morphology of the salivary glands of some other Orthocladiinae species have been considered elsewhere (Mechelke, 1953). The salivary glands of Orthocladiinae have been described as flat sacs composed of three lobes. The glands are paired organs asymmetrically arranged in the larval body. The cell nuclei in the lobes differ in polyteny level, puffing and nucleolus patterns (Mechelke, 1953). The paired salivary glands of *E. subviridis* are sacshaped too. One gland is elongated and the other is ovoid. In *E. subviridis,* the salivary gland cells are radially arranged cells with low ploidy level lying in a semicercle closer to the centre, and the level of chromosome polyteny increases with the distance from the center (fig. 6d–e). An attempt was made to compare the polytene chromosomes from the central and peripheral parts of the salivary gland according to the activities of the nucleoli and Balbiani rings. This seemed reasonable because some Orthocladiinae species were found to differ markedly in the activities of these regions in some of the gland cells. However, no such differences were observed, so far, for *E. subviridis.*

The peripheral gland cells have the highest level of polyteny, and they are, consequently, advantageous for karyological analysis.

It has been reported that ecological and ontogenetic factors affect the structure of the polytene chromosomes (Iliinskaya, 1984; Demin & Iliinskaya, 1986). There are also indications in the literature that compactization of the salivary gland chromosomes in summer may be manifested as loss of the fine structural details. However, we have not observed that this might be the case. Although the *E. subviridis* larvae were fixed in June, the polytene chromosome retained their distinct banding patterns. The discrepance between the observations reported in the literature and ours may be due to the fact that they were made on the represent actives of different Chironomidae subfamilies.

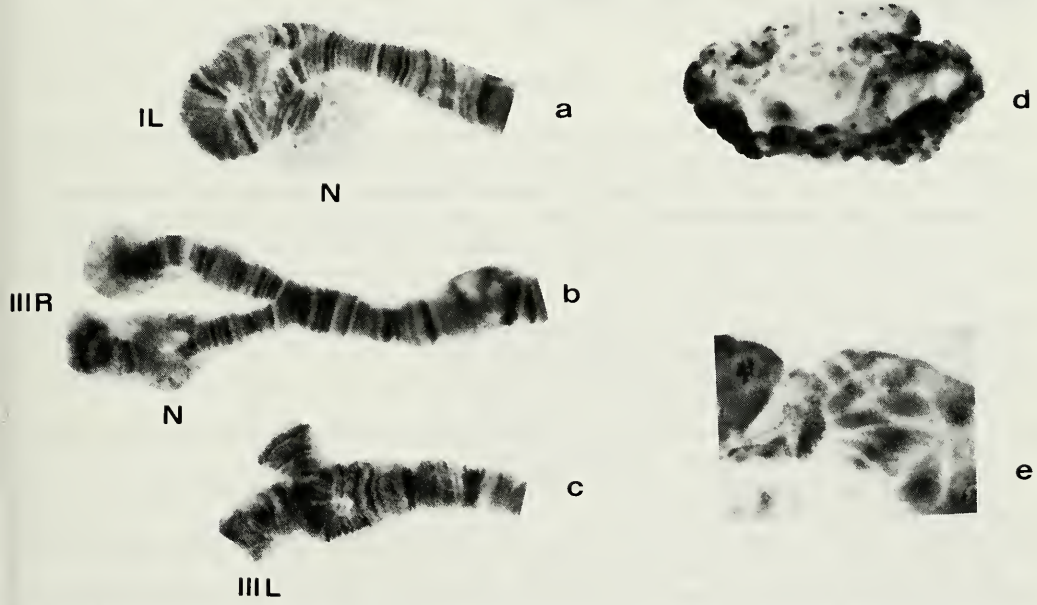

Fig. 6. Arm IL (a), arm IIIR (b), arm IIIL (c) heterozygotes. The morphology of the salivary glands of *Euryhapsis subviridis:* d – magnified part of the gland near the duct; e – general appearance. Cells with nuclei at different levels of polyteny are distinctly seen.

Notes

E. subviridis males are very close in the structure of hypopygium to the North American species *E. illoba* Ol., however they differ from it in the value of AR (AR = 1.06 in *E. illoba).* Individuals of the Far East differ also from the male of the type locality in higher values of AR and LR (AR = 1.25, LR = 0.80 in *E. subviridis* from Austria).

Within the genus *Euryhapsis* the preimaginal stages were previously known only for *E. cilium.* Pupa of *E. subviridis* differs from *E. cilium* in the form of thoracic horn which is bifurcated and totally covered with fine spinules whereas in *E. subviridis* it is simple and covered with spinules only in the upper three quarters. Larvae of *E. cilium* and *E. subviridis* are very similar and they can be distinguished only by certain characters. SI of labrum in *E. subviridis* with 7–9 lobes (more than 10 in *E. cilium),* the seta interna with 5 plumose branches (7 in *E. cilium),* the 6th lateral tooth of mentum is rounded, larger than the 5th and much lower as the other lateral teeth (the 5–6th lateral teeth of mentum of *E. cilium* are of similar shape and size and arranged close to each other).

Comparisions of the major karyotypic features of *E. subviridis* with those of the other Orthocladiinae studied demonstrate similarities with respect to diploid chromosome number (2 n = 6), location of the centromeric region (2 metacentrics, 1 submetacentric) (Michailova, 1976, 1985).

There are some karyotypic features, specific to *E. subviridis* to be noted. The level of chromosome polyteny is higher in *E. subviridis* as compared with that established for the other members of the Orthocladiinae subfamily (Michailova, 1976, 1985). This facilitates chromosome mapping in *E. subviridis.* Another specific feature of the *E. subviridis* karyotype is the unusual telocentric localization of the nucleolar organizer in chromosome I. Futhermore, its polymorphism level is 0.4 heterozygous inversions per individual, higher than reported for the other representatives of the Orthocladiinae subfamily (Michailova, 1985).

It would be of interest to study karyotypes of other species of the genus *Euryhapsis* to provide a broader bases for comparision of the structural organization of chromosomes and also to make judgements about their interrelated evolutionary history.

Literature

Belyanina, S. I., Maximova, F. L., Buchteeva, N. M., Iliinskaya, N. B., Petrova, N. A. & L. A. Chubareva 1983: Karyotype of Chironomus plumosus. – In: *Chironomus plumosus.* Moskow: Nauka p. 61–95

Demin, S. Yu. & N. B. Iliinskaya 1986: Phenotypic variants of the polytene chromosomes of salivary glands in chironomid larvae of natural populations of *Chironomus plumosus* and closely related species. – In: Evolution, speciation and systematics of chironomids. Novosibirsk, p. 107–112

Dévai, G., Wülker, W. & A. School 1983: Revision der Gattung *Chironomus* Meigen (Diptera). IX. *C. balatonicus* sp. n. aus dem Flachsee Balaton (Ungarn). – Acta Zool. Ac. Sci. Hung. **29:** 357–374

Iliinskaya, N. B. 1984: Characteristics of the polytene chromosomes of various degree of compactness in larvae of natural *Chironomus* population. – Cytology **24:** 543–551

Keyl, H. G. 1961: Chromosomenevolution bei *Chironomus.* I. Strukturabwandlungen an Speicheldrüsen-Chromosomen. – Chromosoma **12:** 26–47

Keyl, H. G. 1962: Chromosomenevolution bei *Chironomus.* II. Chromosomenumbauten und phylogenetische Beziehungen der Arten. – Chromosoma **13:** 464–514.

Kiknadze, I. I. & I. E. Kerkis, 1984: Karyotypical characteristic of *Chironomus* f. l. *reductus* with 2 n = 6 from Ob Reservoir. – Cytologia **26:** 735–740

Kiknadze, I. I. & I. E. Kerkis 1986: Comparative analysis of the polytene chromosome banding patterns in sibling species *Chironomus balatonicus* and *Ch. muratensis.* – Cytologia **28:** 430–436

Kuberskaya, E. F. 1974: On the characteristics the karyotype and some special features of the nucleus structure in *Pseudodiamesa nivosa* from the Lake Baikal area. – Cytology **16:** 1426–1432

Kuberskaya, E. F. 1979: Karyotypical characteristic of chironomids subfamily Diamesinae (Diptera, Chironomidae) from Baikal area. – In: Karyosystematic of invertebrate animals. – Leningrad Nauka, p. 47–50

Kuberskaya, E. F. 1984: The morphology of salivary glands and karyotypical characteristic *Diamesa tsutsui* Tok. (Diptera, Chironomidae) from Prymorie. – In: The biology of freshwater animals of the Soviet Far East. Vladivostok, p. 102–106

Kurazhkovskaya, T. N. 1969: The structure of salivary glands in chironomid larvae. – In: Physiology of aqueous organisms and their role in circulation of the organic matter. Leningrad: Nauka, p. 185–195

Martin, J. 1979: Chromosomes as tools in taxonomy and phylogeny of Chironomidae (Diptera). – In: Sæther, O. A. (ed.): Recent development in chironomid studies (Diptera, Chironomidae). Ent. scand. Suppl. **10:** 67–74

Mechelke, F. 1953: Reversible Strukturmodifikationen der Speicheldrüsenchromosomen von *Acricotopus lucidus*. – Chromosoma **5:** 511–543

Michailova, P. 1976: Cytotaxonomical diagnostics of species of the genus *Cricotopus* (Chironomidae, Diptera). – Caryologia **29:** 291–306

Michailova, P. 1980: A review of the european species of genus *Clunio* Haliday, 1855 (Diptera, Chironomidae). – Zool. Anz., Jena **205:** 417–432

Michailova, P. 1985: Cytotaxonomic review of some species of the genus *Orthocladius* Van Der Wulp (Diptera, Chironomidae). – Entomol. Abh. **48:**

Oliver, D. R. 1981: Description of *Euryhapsis* new genus including three new species (Diptera, Chironomidae). – Can. Ent. **113:** 711–722

Oliver, D. R. 1982: *Xylotopus*, a new genus of Orthocladiinae (Diptera, Chironomidae). – Can. Ent. **114:** 167–168

Oliver, D. R. 1985: Review of *Xylotopus* Oliver and description of *Irisobrillia* n. gen. (Diptera, Chironomidae). – Can. Ent. **117:** 1093–1110

Oliver, D. R. & M. E. Roussel 1983: Redescription of *Brillia* Kieffer (Diptera, Chironomidae) with descriptions of Nearctic species. – Can. Ent. **115:** 257–279

Pankratova, V. Ya. 1970: Larvae and pupae of midges of the subfamily Orthocladiinae (Diptera, Chironomidae = Tendipedidae) of the USSR fauna. Leningrad, 344 pp.

Petrova, N. A. 1983: Karyotype and unstable associations of polytene chromosomes in *Syndiamesa nivosa* (Diptera, Chironomidae). – Zool. zhurn. **62:** 69–74

Ryser, N. M., School A. & W. Wülker 1983: Revision der Gattung *Chironomus* Meigen. VII. *C. nudiventris* n. sp. und *C. muratensis* n. sp. Geschwisterarten aus der *plumosus*-Gruppe. – Rev. suisse Zool. **90:** 299–361

Sæther, O. A. 1980: Glossary of chironomid morphology terminology (Diptera, Chironomidae). – Entomol. scand. Suppl. **14:** 51 pp.

Siebert, M. 1979: Two new chironomids (Diptera, Chironomidae) from Germany and Austria. – Aquatic Insects **1:** 165–168

Shilova, A. I. 1976: Chironomids of Rybinsk Reservoir. Leningrad: Nauka, 251 pp.

Wülker, W. & P. Götz 1986: Die Verwendung der Imaginalscheiben zur Bestimmung des Entwicklungszustandes von *Chironomus*-Larven (Diptera). – Ztschr. Morphol. Tiere **62:** 362–388

Wülker, W. & M. G. Butler 1983: Karyosystematics and morphology of northern *Chironomus* (Diptera, Chironomidae): freshwater species with larvae of the *salinarius* type. – Ent. scand. **14:** 121–136

E. A. Makarchenko,
Institute of Biology and Pedology, Far East Science Center, USSR Academy of Sciences, Vladivostok, USSR,
I. I. Kiknadze, I. E. Kerkis,
Novosibirsk University,
Novosibirsk, USSR