

Cytotaxonomy and Morphology of Chironomid Larvae (Diptera, Chironomidae) in Armenia

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Abstract—In the study of chironomids in Armenia several species of *Orthoclaadiinae* subfamily of *Cricotopus* genus, *Diamesinae* subfamily of *Diamesa* genus, and *Chironominae* subfamily of *Chironomus* genus, have been identified. In the *Cricotopus* genus two sibling species were found, not distinguishable by larval morphological features, but clearly distinct cytogenetically.

Keywords—Armenia, Chironomidae, karyotype, larval morphology.

I. INTRODUCTION

CYTOGENETIC studies on the description of karyotypes and morphology of the chironomid larvae of the Armenian fauna were started in comparatively recent times [5, 6, 13, 14]. In previous works we have encountered some difficulties that were associated with the uniqueness of the local fauna of Armenia. It is in Armenia that most chironomids belong to the *Diamesinae*, *Pseudodiamesinae*, *Orthoclaadiinae* subfamilies, species of which are extremely difficult to be identified by larvae. Known keys of chironomid by larval karyotypes and morphology [8, 9] and polytene chromosome atlases published [10] were not sufficient to work with the little-known fauna. This article continues the description of karyotypes and morphology of chironomid larvae in Armenia. This collection contains difficult for identification larvae of the *Orthoclaadiinae* and *Diamesinae* subfamilies. Also two species of the *Chironominae* subfamily (*C. riparius*, *C. barbipes*) were found, karyotypes of which are well studied from Palearctic.

II. MATERIALS AND METHODS

The material was collected from reservoirs of different regions of Armenia from 28.07.2010 to 16.09.2010. Spot sampling were made in reservoirs near the following settlements: two collections in Gegharkunik region - from a tributary of the Vardenis River near Vardenik village and near Yeranov village in a small stagnant reservoir; two collections in Kotayk region - Ddmashen village from a stream and near

Arzni village from a dirty tributary of the Hrazdan River; near the Parz Lich Lake - from a mountain stream; three collections in Ararat region - from Hovtashen village in an irrigation canal almost with stagnant water, Zavashat village from the Azat River and in Urtsadzor village from Vedi River; and near Kuchak village from a tributary of Azat Reservoir. Elevation of the location is from 842m to 1910m, pH environment ranging from 5.5 to 7.0. Larvae were collected by a net with the ring of 15 cm in diameter, then washed at collection site using soil sieve with mesh diameter of 0.5 mm. The average flow velocity was from 2 m/sec to the complete absence of flowing. Collections were made at depths of 0.1 - 0.5 m. The substrata, inhabited by the larvae, were gray silts on the rocky underlay or just a rocky bottom. From each collection 3 - 16 larvae has been processed. The collected material was fixed at collection site in Carnoy's fixative (3:1 ethanol 96%: ice acetic acid).

Morphological and karyological preparations were made for each larva. Morphological preparations consisted of separate parts of the head capsule and the last 7 - 9 abdominal segments. Making of morphological preparations was carried out according to known methods [1, 12, 15].

To make the karyological preparations salivary glands were isolated in a drop of lactic acid, stained for 20 minutes with 2% aceto-orceine solution, and after the second maceration in 45-60% lactic acid, cells were separated from secretion. Temporary crushed preparations were made by routine method [2].

III. RESULTS

The characteristics of the morphological and karyological features of the chironomid species studied are given below.

Orthoclaadiinae Subfamily.

Cricotopus Genus (Van der Wulp, 1874).

Cricotopus f. l. ex. gr. bicinctus (type species - *C. bicinctus* Meigen, 1818).

Larva (Fig. 1 a-d). The larva is 8 mm in length. The head capsule is dark. Mandible is with 5 external equally dark teeth, the first tooth much longer than others. Mentum with single median and 5 pairs of lateral teeth smoothly coming down, and the fifth pair is very small. Premandibule segment is distally slightly splitted into two broad teeth. Antenna is 5 segmented, its seta is longer than flagellum. There is a large ring organ at the base. Length ratio of antennal segments is 20:5:2:2:1. Antennal ratio - 2.

Karyotype (Fig. 1 e, f). $2n = 4. I > II$. Both chromosomes are metacentric. Centromeres are dark, heterochromatic, tend to ectopic conjugation. They are in the middle part of each

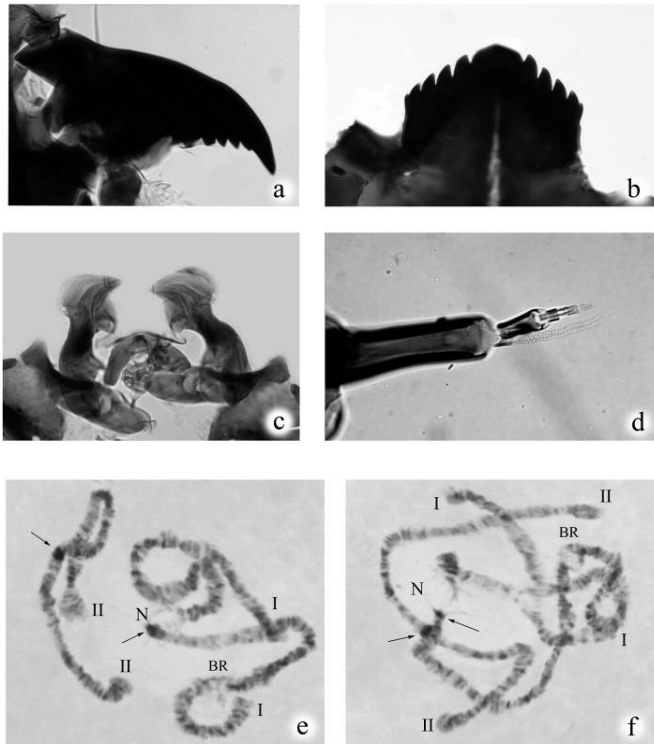
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chromosome. Near the centromere of chromosome I nucleus (N) is located. It is large, well developed. In the same chromosome there is a Balbiani ring (BR). Both ends of chromosomes II are puffed.



Notations: a - mandible, b - submentum, c - premandible, d - antenna, e, f - karyotype. IA, IB, etc. - the arms of the chromosomes; N - nucleolus, BR - Balbiani rings, P - puffs; arrows denote the centromeres.

Fig. 1. Morphology and karyotype for *Cricotopus f. l. ex gr. bicinctus*.

The type species *C. bicinctus* was discovered more than 20 years ago [16], the karyotype was published one year later [10]. The species has $2n = 6$. Our species, which we defined as *C. f. l. ex gr. bicinctus* species has $2n = 4$. Perhaps the different diploid numbers of chromosomes ($2n = 6$ and $2n = 4$), centromeres tending to conjugation, well-developed N and BR, compared with karyotype *C. bicinctus*, indicate that the species described is separate, closely related to morphologically similar *C. bicinctus* species.

Cricotopus f. l. ex gr trifascia sp.1 (type species - *Cricotopus trifascia* Edwards, 1929).

Larva (Fig. 2 a-d). The larva is 8 mm in length. Mandible is with 4 external equally dark teeth, the first tooth is a little longer than others. Bristle under the teeth is shapely. Mentum has a median tooth, characteristic for the group, with two pairs of notches on the sides, making it look like five joined teeth. There are four pairs of lateral teeth, of which the most extreme ones are hardly noticeable. Premandibule is distally not split. Antenna is 5 segmented; there are two large ring organs at the base. Seta reaches end of the third segment. Length ratio of antennal segments is 20:5:2:1:1. Antennal ratio - 2.

Karyotype (Fig. 2e). $2n = 6$. According to length the relationship between three chromosome pairs is the following: $IAB = IICD > III EF$. As a result of preliminary mapping the chromosomes was divided: IAB - in 21 regions, IICD - in 17 regions, III EF - in 19 regions. Centromeres are dark, heterochromatic. In chromosome I (reg. 11) and II (reg. 9) they are located medially, whereas in the chromosome III centromere is localized in the region near to telomere (reg. 1). The species is binucleolar. In the karyotype, there are two well-developed N. N1 is in the reg. 16 of chromosome I, N2 is located in the reg. 1 of chromosome III near the centromere, almost terminally. BRs are localized in the reg. 2 of chromosome I and reg. 5 of chromosome III. In chromosome II there are large puffs (reg. 3, 6, 10, 16). In the karyotype rearrangements were not found.

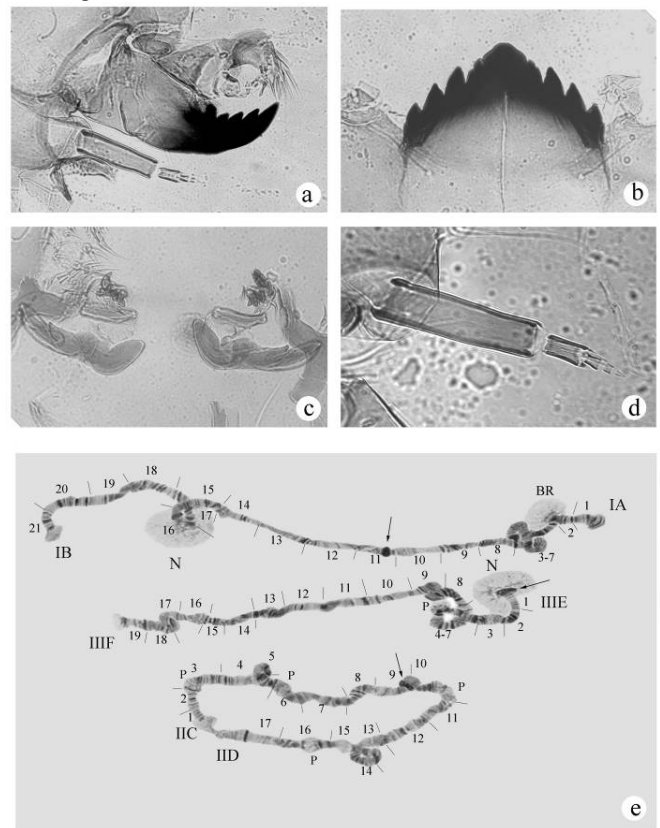


Fig. 2. Morphology and karyotype for *Cricotopus f. l. ex gr. trifascia sp. 1*: notations are the same as in Fig. 1.

Cricotopus f.l. ex. gr. trifascia sp.2.

Larva (Fig. 3 a-d). The larva is 8 mm in length. Mandible is with four dark teeth, the first tooth is slightly bigger than the others. Seta under the teeth is lanceolate, curved, not reaching the first true tooth. Mentum is arranged similar to the previous species. Median tooth bears two pairs of notches. Four pairs of teeth located on each side of mentum, the most extreme teeth are negligible. Premandibula has a large rounded tooth. Antenna is 5 segmented; there are two large ring organs at the base. Length ratio of antennal segments is 44:14:5:3:3, antennal ratio - 1.7.

Karyotype (Fig. 3e). $2n = 6$. Chromosomes are indicated IAB, IICD, IIIEF; as a result of preliminary mapping they were divided: IAB - in 17 regions, IICD - in 20 regions, IIIEF - in 20 regions. Centromeres do not differ from general bands in morphology. We supposedly have marked them in chromosome I (reg. 11), in chromosome II (reg. 9), in chromosome III (reg. 6). In reg. 6 of chromosome I there is a large, well-developed N. Neither puffs nor BRs were found in the karyotype.

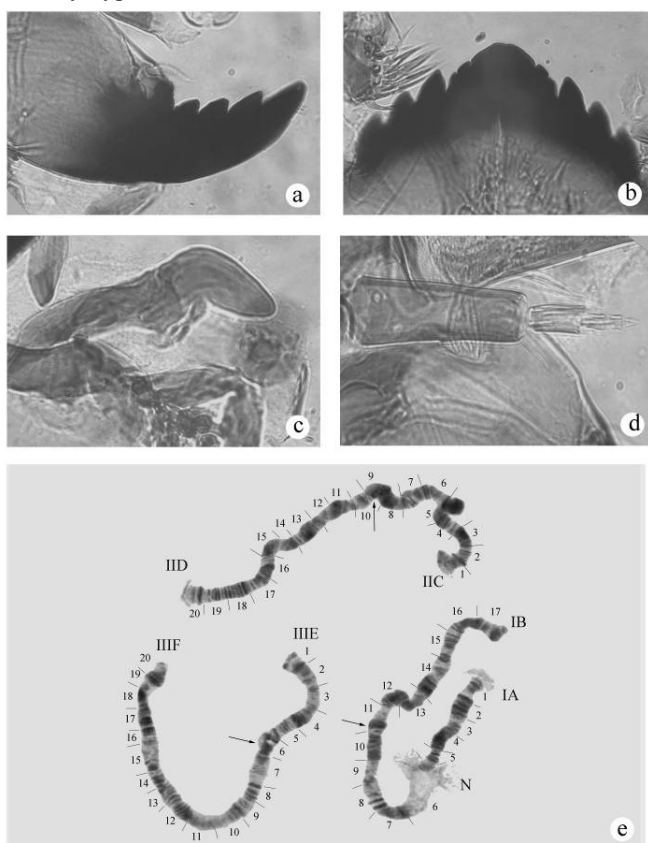


Fig. 3. Morphology and karyotype for *Cricotopus f. l. ex gr. trifascia sp.2*: notations are the same as in Fig. 1.

Diamesinae Subfamily Edwards, 1929.

Diamesa Meigen, 1835.

Diamesa bertrami, Serra-Tosio, 1968.

Larva (Fig. 4 a-d). Mandible has 5 teeth arranged like a fan. Their relative sizes gradually decreased from the first to the fifth one. Seta under the teeth is slender, up to the fourth external tooth. Mentum has a bifurcated median tooth, and 10 pairs of lateral ones. The first and second pairs of lateral teeth are about the same height with a median tooth. Premandible is distally divided into 8 rounded teeth, they are arranged in the form of a teaspoon. The antenna consists of four segments, the third segment is annulated. At the bottom there is a large ring organ. Antennal seta reaches the end of the third segment. Length ratio of antennal segments is 40:10:10:5. Antennal ratio -1.6.

Karyotype (Fig. 4e) $2n = 8$. The arms are marked: IAB, IICD, IIIEF and IVG. As a result of ectopic contacts between

telomeres, large chromosomes of this species often form a chain. N is localized near to telomere in the IVG chromosome. Dark band, located next to the nucleolus may serve as a species marker. Dark heterochromatic bands, which we consider centromeres are marked by arrows in the photo. Three BRs are located in the chromosome IAB, the fourth and fifth BRs - in the chromosome IIIEF.

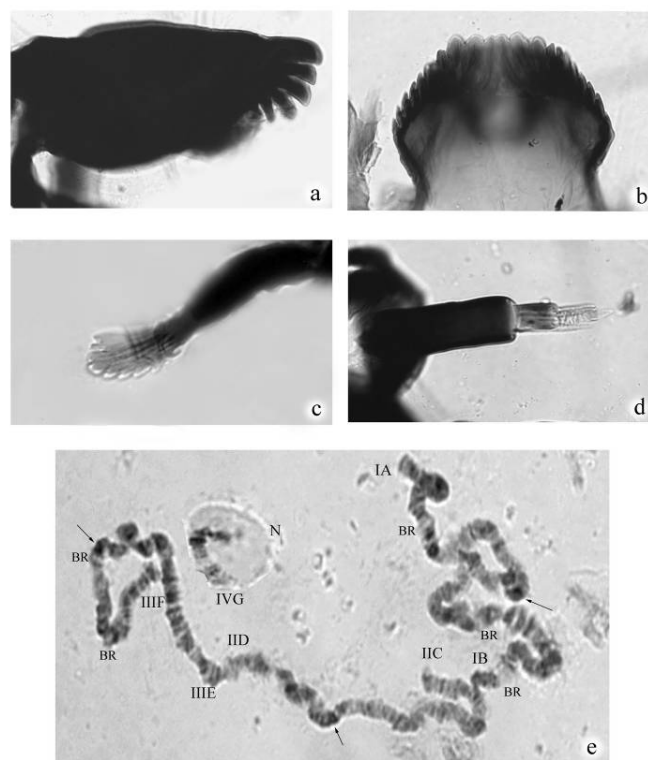


Fig. 4. Morphology and karyotype for *Diamesa bertrami*: notations are the same as in Fig. 1.

Diamesa aberrata Lündbeck, 1898.

Larva (Fig. 5 a-d). Larva 10 mm long. The head capsule is dark. Mandible is with 5 external teeth, the first two larger than the others. Mentum with lower and flat median tooth, and it is two times wider than the first lateral pair. 9 pair lateral teeth come down domically. Premandible is distally splitted into six teeth rounded apically, arranged like an asymmetric fan. Antenna is 4 segmented, length ratio of antenna segments is 46:10:5:4. Antennal ratio - 2.4.

Karyotype (Fig. 5e). $2n = 8$. The elements of the karyotype are marked according to Mikhailova [10]. Large N is in the region near to telomere in the chromosome IVG. Supposed centromeres are indicated by the arrow in the figure. Two BRs are in chromosome II.

Chironominae Subfamily Macquart, 1838.

Species belonging to the *Chironominae* subfamily, are well studied, and are easily identified at the larval stage. Therefore, the description of the morphology is not given here.

Glyptotendipes Kieffer, 1818.

Karyotype of *Glyptotendipes barbipes* (Staeger, 1839) (Fig. 6). One larva was found. $2n = 8$. IAB, IICD, IIIEF, IVG.

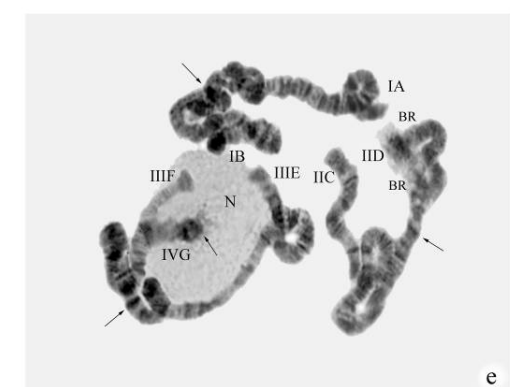
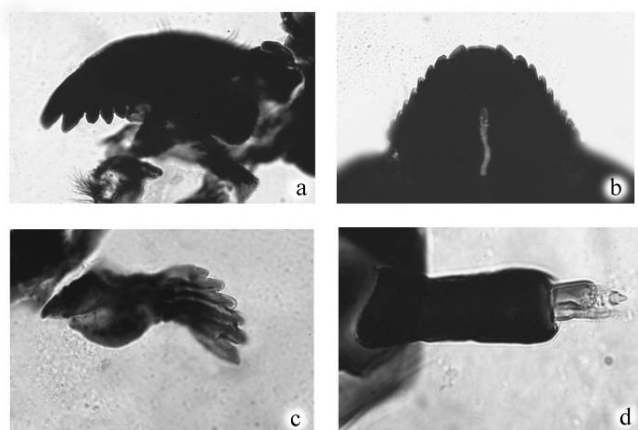


Fig. 5. Morphology and karyotype for *Diamesa aberrata*: notations are the same as in Fig. 1.

The karyotype is homozygous, significant deviations from the chromosomes of the karyotype, taken as a standard, are not found [8, 9, 10]. Centromeres appear as large heterochromatic blocks. N-s are active, they look like developed puffs. One BR is localized in chromosome II, and the other one - in chromosome IV. In chromosome I a large puff is active.

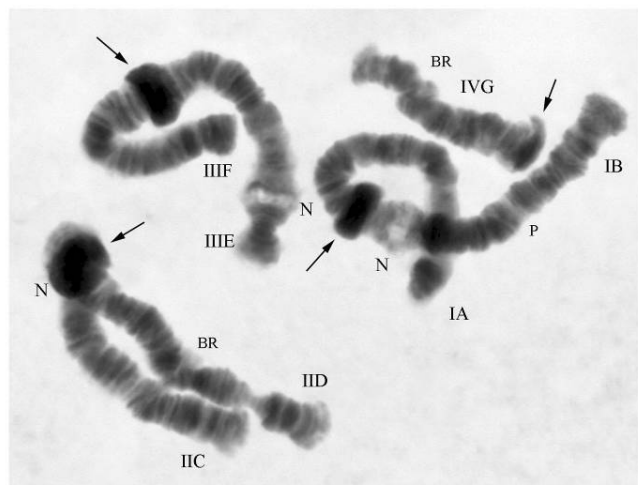
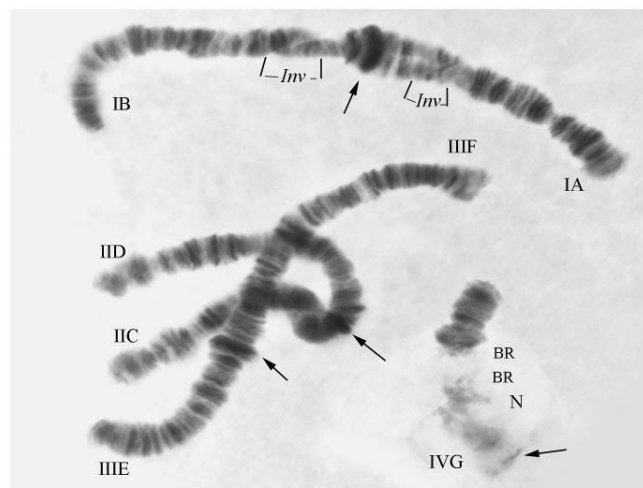


Fig. 6. Karyotype for *Glyptotendipes barbipes*: notations of karyotype elements are the same as in Fig. 1.

Chironomus Meigen, 1803.

Karyotype of *Chironomus riparius* (Meigen, 1804) (Fig. 7). $2n = 8$. IAB, IICD, IIIEF, IVG. The regions near centromeres are much less pronounced than in previous species. However, the centromeres significantly stand out from general bands [11]. Chromosome IVG is physiologically active; there are two BRs and well-developed N. In the karyotype of the individual studied, heterozygous rearrangements in chromosome I are found. They touch the arm A in reg. 14-16, and the arm B in reg. 21-25.



Notation: Inv – regions of inversions.

Fig. 7. Karyotype for *Chironomus riparius*: notations of karyotype elements are the same as in Fig. 1.

IV. DISCUSSION

Thus, the results of the next phase of the study of fauna and karyotypes of chironomids from reservoirs in Armenia are given. Seven species of three subfamilies - *Orthocladinae*, *Diamesinae* and *Chironominae* - are described. *Glyptotendipes barbipes* species is recorded for the first time for the area of Lake Sevan basin. A single finding is explained by the fact that for *G. barbipes* study area is located on the border of range, separated from the rest of the range by mountain chain and two seas. On the other hand, this species prefers slow eutrophic reservoirs, which are few in Armenia. It is noteworthy that *G. barbipes* is considered a highly polymorphic by chromosomal inversions, rearrangements of all chromosome arms are found [9]. On average, each individual in the population carries at least one aberration, whereas the individual from Gegharkunik has a standard karyotype. Probably, on the border of range environmental conditions limit the possibility of inverted and heterozygous variants, and individuals with no aberrations have advantage, because in the middle of the range there are more aberrations than at the borders [3].

Finding of species *Chironomus riparius* in this collection of Armenia is not an exceptional event, as in the valley of the Hrazdan River it is common [14]. However, it is worth noting that this species is characterized by low level inversion polymorphism for macroinversions at a fairly large variety of

them [7]. The individual described (one out of 15) carries two inversions in karyotype, although small in size. This individual was found in a channel with very slow current at Hovtashen village. However, we are not inclined to consider this single case as a result of any human impact and attribute it to natural fluctuations of species karyofund.

Species of the *Cricotopus* genus in the larval stage are almost not differentiated by morphological features. Even the classic work of Hirvenoja [4] has not made it possible to solve a number of systemic problems in identifying *Orthoclaadiinae*. A good illustration of this problem is a case of finding of two species of the *Cricotopus* genus, from the same group, presented by us in this work. They were found in the same habitat: in Vedi River at Urtsadzor village. With similar morphological features, they have entirely different karyotypes. Despite the impossibility of identifying them to species (only to the group *trifascia*), we can confidently talk about different species. Thus, we are dealing with sibling species, sympatrically coexist in the same community of hydrobionts.

The individual, which we previously identified as *Cricotopus f. l. ex. gr. bicinctus*, has larval morphological features that can be called the most specific to the larvae of the *Cricotopus* genus. However, according to the structure of the karyotype the larva could not be attributed to the known species of the genus [10], and we did not find similarities with karyotype of *C. bicinctus*. Probably, this individual can pretend to be a separate species.

Karyotypes in the *Diamesa* genus have a very similar organization. As a rule, $2n = 8$, three pairs of chromosomes are long, and the fourth pair is very short, with a terminally located nucleolus. Chromosomes in *Diamesa* are thin and long, closely associating with the karyotheca, and they are often uncomfortable subject in comparison with the representatives of the *Chironominae* subfamily. For this reason karyotaxonomy of *Diamesa* is at the development stage. As a rule, $2n$ and the most common characteristics of the karyotype are indicated in publications [10]. Despite of several attempts to find homologous band sequences for different species of the *Diamesa* genus, the establishment of relationships between them is still impossible. In this paper we confined ourselves to a description of the studied karyotypes of *D. bertrami* and *D. aberrata* without interspecific analysis.

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REFERENCES

[1] Chernovskii A.A., 1949. Key of mosquito larvae of the Tendipedidae family (Chironomidae). Keys to the fauna of USSR. Moscow - Leningrad, Izd. USSR Academy of Sciences, v. 31, 186p.

[2] Chubareva L.A., Petrova N.A., 1982. Cytologic method for the chromosome analysis in Chironomidae // Tool for the Study of chironomids. - Dushanbe: Donish, p. 64-73.

[3] Dobzansky Th., 1970. Genetics of the Evolutionary Process. - New York, London: Columbia University Press, 506 p.

[4] Hirvenoja M., 1973. Revision der Gattung *Cricotopus* van der Wulp und ihrer Verwandten (Diptera, Chironomidae). Ann. Zool. Fenn. 10: 1-363.

[5] Kachvoryan E.A., Hovhannisyan V.S., Petrova N.A., Zelentsov N. N., 2007. The Species Composition of Chironomids and Black Flies (Diptera: Chironomidae, Simuliidae) in Hrazdan River in Armenia and hydrochemical characteristics of the reservoir // Entomological Review, LXXXVI, № 1, p. 73 - 82.

[6] Karageuzyan K. G., Chubareva L. A., Kachvoryan E. A., Adler P. H., Petrova N. A., et al., 2003. Ecological conditions of Hrazdan River (Armenia). Report 1 // Bulletin of the International Academy of Ecology and Life Safety. V. 8, № 4. P. 30 - 34.

[7] Kiknadze I.I., Gunderina L.I., Filippova M.A., Seraya E.I., 1988. Chromosomal polymorphism in laboratory and natural populations of *Chironomus thummi* Kieff. // Genetics. V. 24, № 10. P. 1795-1805.

[8] Kiknadze I.I., Istomina A.G., Gunderina L.I., Salova T.A., Aimanova K.G., Savvinov D.D., 1996. Karyofunds of chironomids of permafrost of Yakutia: Chironomini Tribe / Ed. Gruzdev A.D. - Novosibirsk: Nauka, 166p.

[9] Kiknadze I.I., Shilova A.I., Kerkis I.E., Shobanov N.A., Zelentsov N.I., Grebenyuk L.P., Istomina A.G., Prasolov V.A., 1991. Karyotypes and morphology of larvae of the Chironomini tribe. Atlas.- Novosibirsk: Nauka, 114p.

[10] Michailova P., 1989. The polytene chromosomes and their significance to the systematic of the Chironomidae family, Diptera // Acta Zool. Fentica, V.186. P. 1-107.

[11] Michailova P., Petrova N., Sella G., Ramella L., Todorova D., Zelano V., 1996. Cytogenetic characteristics of a population of *Chironomus riparius* Meigen 1804 (Diptera, Chironomidae) from a Po river station // Genetica, V. 98, P. 161 - 178.

[12] Pankratova V.Ya., 1970. Larvae and pupae of mosquitoes of the Orthoclaadiinae subfamily of the USSR fauna (Diptera, Chironomidae = Tendipedidae) / Science (Leningr. Dep.), Leningrad, 344p.

[13] Petrova N.A., Chubareva L.A., Kachvoryan E.A., 2004. Karyotypic characteristics and Inversion Polymorphism in *Prodiamesa olivacea* Mg. (Diptera, Chironomidae, Prodiamesinae) from Armenia // Bulletin of the International Academy of Ecology and Life Safety. V. 9, № 3, P. 26 - 29.

[14] Petrova N.A., Zhirov S.V., Zelentsov N.I., Kachvoryan E.A., 2011. On the fauna of chironomids (Diptera, Chironomidae) of the Hrazdan River basin (Armenia) // Zool. Journal. Vol. 4. P. 445-451.

[15] Pinder L. C. V., 1983. The larvae of Chironomidae (Diptera) of the Holarctic region - Introduction // Ent. Scand., Supp. 19. 7 – 10pp.

[16] Shilova A.I., Zelentsov N.I., 1988. On the fauna of chironomids of the Sevan Lake (Diptera, Chironomidae) // Biol. interior. waters. Inf. Bull. P. 48-52.