Karyotype structure and polymorphism peculiarities of Chironomus nuditarsis Keyl, 1961 (Diptera, Chironomidae) from natural populations of North Caucasus

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A B S T R A C T

The study presents data on the karyotype characteristics and features of chromosomal polymorphism of Chironomus nuditarsis Keyl, 1961 (Diptera, Chironomidae) from seven natural populations of Caucasus (Northwest, Central and East Caucasus). We found 16 banding sequences in the Caucasian populations. We observed inversion polymorphism almost in all chromosome arms except for arms C and E. The genetic distances between all the studied populations of Ch. nuditarsis were calculated using Nei criteria (1972). In spite of relative geographic proximity, the genetic distances between populations of the Caucasus are quite big, and they do not form a single cluster of Caucasian populations. The population of the Northwest Caucasus goes to European cluster; the populations of Central and East Caucasus form their own separate clusters. The principal component analysis (PCA) shows the similar picture. We suggest that such a clear separation of Caucasian populations in distinct clusters is a result of differences of collection sites in terms of geography and climate (complex diverse terrain and microclimate conditions). Four of the Caucasian populations do not follow Hardy-Weinberg expectation. In two populations, there being a marked deficiency of heterozygotes in arms B, F and G. In two other populations, there being a marked excess of heterozygotes in arms B and G. One can suggest that observed pictures could be a reflection of multi-directional selection of heterozygotes in different populations. The populations of Ch. nuditarsis from different parts of the Caucasus possibly diverged from each other at the level of subspecies. All the obtained data are indicative of the complex genetic structure of Caucasian populations of Ch. nuditarsis and total complexity of microevolution processes occurring in the Caucasus region.

1. Introduction

According to the Fauna Europaea web source (Pape and Beuk, 2016) the species is known in Northern (British Isles, Danish mainland, Norway), Western (except for Belgium), Southern (Spanish mainland, Italian mainland) and Eastern Europe (Poland, Czech Republic, Slovakia, Hungary, Bulgaria and Northwestern European Russia (Pskov oblast)). According to Kiknadze et al. (2016), the species is known from Belgium (Natural reserve Waelen hoek), Western Siberia and Republic of Altai. Keyl (1961, 1962) described the karyotype and polytene chromosomes banding sequences of Ch. nuditarsis (1961) from German populations. Earlier a high level of chromosomal polymorphism (inversions, reciprocal translocations, DNA-knobs) was observed in West and East European populations of Ch. nuditarsis (Rosin and Fischer, 1965, 1966, 1972; Fischer, 1978; Fischer and Rosin, 1967; Fischer and Tichy, 1980; Michailova, 1989; Zhirov and Petrova, 1993; Petrova et al., 2000; Polukonova et al., 2005). Kiknadze and coauthors (Kiknadze et al., 2006) presented a comparison of karyotypes and chromosomal polymorphism between European and Asian Ch. nuditarsis populations. Michailova (1989) considered that there are two cytological races of Ch. nuditarsis in Bulgaria differing by the size of centromeric bands. Also, Michailova et al. (2002) provided some information on cytology of Ch. nuditarsis in Polish populations.
shown that Ch. nuditarsis and Ch. plumosus is very close in the sense of phylogeny (Kiknadze et al., 2006).

Polukonova and Karmokov (2013) previously described the karyotype and chromosomal polymorphism of Ch. nuditarsis from three Central Caucasian populations.

The aim of the work was to present the description of karyotype structure and polymorphism peculiarities of Ch. nuditarsis from seven new Caucasian populations. In addition, it was also very important to compare the chromosomal polymorphism characteristics of Ch. nuditarsis from the Caucasus with earlier studies.

2. Materials and methods

We used fourth instar larvae of Chironomus in the karyological study. We provide the collection sites and abbreviations of earlier studied populations (Kiknadze et al., 2006) in Table 1. The Caucasus region (Russian Federation) served as larvae collection sites and included two sites from Krasnodar Krai, six sites from the Republic of Kabardino-Balkaria and two sites from the Republic of Dagestan (Table 2). Collection sites are marked on the map with dark dots (Fig. 1). The geographic division of the Caucasus is due to the Gvozdetskii (1963). The area to the west of Mount Elbrus is considered as the Northwest Caucasian. The area between Mount Elbrus and Mount Kazbek is considered as the Central Caucasian. The area to the east of Mount Kazbek is considered as the East Caucasian. We are located in the coastal zone of the Caspian Sea.

The head capsule and body of up to 60 larvae were slide mounted in Fora-Berlese solution for morphological identification. We studied the karyotype and chromosome polymorphism in 336 larvae from the Caucasus region.

We fixed the larvae for karyological study in ethanol-glacial acetic acid (3:1). The slides of the chromosomes were prepared using the ethanol-orcein technique (Dyomin and Iliynskaya, 1988; acetic acid (3:1). The slides of the chromosomes were prepared using the ethanol-orcein technique. The banding sequences were desigated per the accepted convention specifying the abbreviated name of the species, symbol of chromosome arm, and sequence number as in ndt A1, ndt A2, etc. (Keyl, 1962; Wülker and Klötzi, 1973). We performed the identification of chromosome banding sequences for arms A, E and F using the photomaps of Kiknadze et al. (2006, 2016) in the system of Keyl (1962) and chromosome mapping for arms C and D as per Kiknadze et al. (2006) in the system of Dévai et al. (1989).

We studied the chromosome slides using Carl Zeiss Axio Imager. A2 microscope and performed the statistical data processing using software packages PAST 3.18 (Hammer et al., 2001), GenALEX 6.503 (Peakall and Smouse, 2006, 2012) and STATISTICA 10 (StatSoft).

We used such parameters of chromosomal polymorphism characteristics for comparison as a percentage of heterozygous larvae and the number of heterozygous inversions per larvae.

We calculated the genetic distances between populations, according to Nei criteria (Nei 1972) using Chironomus 1.0 software (Kazakov and Karmokov 2015) based on original data along with Kiknadze et al. (2006) and Polukonova and Karmokov (2013) research results.

3. Results

We attributed the larvae of Chironomus in the studied sites to Ch. nuditarsis by both morphological and chromosomal characteristics. The morphological larval characters of Ch. nuditarsis from the Caucasian sites are similar to those previously described for this species (Webb and Scholl 1985; Polukonova, 2005).

3.1. Karyotype of Ch. nuditarsis from the Caucasus region

The diploid number of chromosomes in Ch. nuditarsis karyotype is 2n = 8, chromosome arm combination is AB, CD, EF, and G (the “thummi” cytocomplex) (Fig. 2). The chromosomes AB and CD are metacentric, EF is submetacentric, and G is telocentric. There is one nucleolus (N) in arm G. There are two Balbiani rings (BR) in the karyotype: one in arm B and one in arm G (Fig. 2).

3.2. Banding sequences and chromosomal polymorphism of Ch. nuditarsis from the Caucasus region

Previously, Kiknadze et al. (2006, 2016) listed 16 banding sequences and two sequences with giant DNA-knobs in banding sequences pool of Ch. nuditarsis. Polukonova and Karmokov (2013) also described new sequence ndt F2 in banding sequences pool of three Central Caucasian populations. In the studied populations, 14 of those sequences are present, and three banding sequences have been found for the first time, providing totally 17 banding sequences in the Caucasian populations (Table 3).

Arm A has five banding sequences: ndt A1, ndt A2, ndt A3, ndt A4 and ndt A7 (Figs. 2–3, Table 3). The banding sequence ndt A1 and zygotic combination ndt A1.1 were predominant in populations of Central and Eastern Caucasus (Table 3, 4). In populations

Table 1

| Locality               | Population abbreviation | Collection sites                           | Collection date | Number of specimens |
|-----------------------|-------------------------|--------------------------------------------|----------------|---------------------|
| **European population** |                         |                                            |                |                     |
| EU-BE-WA              | Belgium, Nei, Waaehoek Nature Reserve, clay-pits | 09.03.94 | 44 |
| EU-BG-CD              | Bulgaria, Montana, Rasovo Lom, Lomsko blato, Saraja, Cholochene, Volugaj, Kalimok, Stovo | 16.05.02–03.07.03 | 16 |
| EU-IT-TU*             | Italy, City of Turin, pond in the Botanic garden | 16.06.96 | 26 |
| SB-NSK-KR             | Novoshirskaya Oblast, pond beside Karpaisk river | 13.05.96 | 19 |
| SB-RAL-Te             | Republic of Altai, Tenginsko lake | 05.1997 | 35 |

* Data provided per Petrova et al. (2000).
of Northwest Caucasus, the banding sequences ndt A1, ndt A2 and the heterozygous zygotic combination ndt A1.2 were predominant. The banding sequence ndt A7 is new for the species and described for the first time (Fig. 3, Table 3, 4). It differs from the sequence ndt A1 by one short inversion step that involves regions 7d-4d 2 h-3i 12c-11a:

ndt A7 1a-2c 10a-e 7d-4d 2 h-3i 12c-11a 8a-9d 4a-c 2 g-d 9e 13a-19f 28de C

The banding sequence ndt A7 was found only in one of the populations of the Eastern Caucasus (EC-SAM-CR) with extremely low frequency (ndt A7 – 0.007) and only in the heterozygous state (ndt A1.7 – 0.014) (Table 3, 4).

Arm B has two banding sequences: ndt B1 and ndt B2 (Fig. 2; Table 3, 4). The banding sequence ndt B1 was predominant in most of the studied Caucasian populations (Table 3, 4). The zygotic combination ndt B1.1 was predominant in three populations of Central Caucasus (CC-NLC-P, CC-GHN-LS and CC-VG-LA). The heterozygous

Table 2
Collection sites and number of analyzed Ch. nuditarsis larvae from the Caucasus region.

| Localities                  | Population abbreviation | Collection sites                                                                 | Collection date | Number of specimens |
|-----------------------------|-------------------------|---------------------------------------------------------------------------------|-----------------|---------------------|
| Northwest Caucasus          | NWC-PSB-FC              | 44°3.8200’ N; 40°45.6170’ E, Krasnodar Krai, small creek under the forest canopy beside the fish ponds, ca 2 km south of the Psebai settlement, altitude ca 690 m a.s.l., max. depth 0.5 m. | 15.06.13        | 38                  |
|                             | NWC-MEZ-BP              | 44°12.4760’ N; 39°58.5040’ E, Krasnodar Krai, “Bolnichnii” pond, ca 1 km northeast of Mezmai settlement, altitude ca 760 m a.s.l., max. depth 1.5 m, water surface 1015 m². | 11.10.19        | 36                  |
| Central Caucasus            | CC-ZAR-^P               | 43°56.2860’ N; 43°51.8960’ E, Republic of Kabardino-Balkaria, puddle in the bed of drained pond, beside the Zarechnii settlement, altitude ca 280 m a.s.l., max. depth 0.5 m. | 18.02.09        | 25                  |
|                             | CC-NLC-^P               | 43°28.5140’ N; 43°36.4050’ E, Republic of Kabardino-Balkaria, puddle in the bed of drained pond, in the city of Nalchik, beside “Nart” hotel, altitude ca 280 m a.s.l., max. depth 1 m. | 05.02.09        | 25                  |
|                             | CC-SKL-^P               | 43°14.9040’ N; 42°38.2120’ E, Republic of Kabardino-Balkaria, small pond, in the boundaries of Elbrus settlement, beside “Sokol” holyday base, altitude ca 1790 m a.s.l., max. depth 0.7 m, water surface 12.4 m². | 27.08.08        | 20                  |
|                             | CC-GHN-L                | 43°32.1810’ N; 43°11.3610’ E, Republic of Kabardino-Balkaria, small lake, ca 2.3 km southwest of Ghankhoteko settlement, altitude ca 808 m a.s.l., max. depth 1.3 m, water surface 1176 m². | 15.06.12        | 56                  |
|                             | CC-KMS-L                | 43°19.5430’ N; 42°48.3220’ E, Republic of Kabardino-Balkaria, western shore of Komsomolskoe lake, altitude ca 1454 m a.s.l., max. depth 2 m, water surface 16208 m². | 19.04.16        | 61                  |
|                             | CC-VG-LA                | 43°13.517’ N; 43°33.7480’ E, Republic of Kabardino-Balkaria, a puddle beside the southern shore of Verkhnee Goluboe lake, altitude ca 915 m a.s.l., max. depth 0.2 m. | 04.09.16        | 36                  |
| Eastern Caucasus            | EC-SAM-CR               | 41°51.843’ N; 48°33.576’ E, Republic of Dagestan, Magaramkentsky District, ca 2 km northwest of Primorsky settlement, a puddle feed by water from the fishpond, altitude ca ~25 m b.s.l., max. depth 0.3 m. | 19.05.14        | 74                  |
|                             | EC-AGR-CHT              | 43°47.557’ N; 47°31.656’ E, Republic of Dagestan, Kizlyar’sky District, ca 8.5 km southeast of Staroterechnoe settlement, “Chakannii” checkpoint of Dagestan Nature Reserve, left shore of Kulyakinsky channel, altitude ca ~25 m b.s.l., max. depth 1 m. | 18.05.17        | 35                  |

- Data provided per Polukonova and Karmokov (2013).

Fig. 1. Collections sites of Ch. nuditarsis in Caucasus region. Collection sites are marked with dark dots. Triangles indicate the Elbrus and Kazbek mountains, along which the geographical division of the Caucasus is carried out.
combination ndt B1.2 was predominant in one population of the Northwest Caucasus (NWC-PSB-FC) and two populations of Central Caucasus (CC-ZAR-P and CC-SKL-P). The zygotic combination ndt B2.2 was predominant in one population of the Northwest Caucasus (NWC-MEZ-BP) and two populations of Eastern Caucasus. We found zygotic combinations ndt B1.1 and ndt B1.2 with the equal frequencies in one populations of Central Caucasus (CC-KMS-L).

**Arm C** was monomorphic with banding sequence ndt C1.1 (Fig. 2, Table 3, 4).

**Arm D** has two banding sequences: ndt D1 and ndt D2 (Figs. 2, 4). The banding sequence ndt D1 and zygotic combination ndt D1.1 were predominant in most of the Caucasian populations (Table 3, 4). The banding sequence ndt D2 is new for the species and described for the first time (Fig. 4, Table 3, 4). It differs from ndt D1 by one simple inversion step that involves regions 9a-10a 13a-11a 3 g-2hg:

\[\text{ndt D2} 1a-2f 9a-10a 13a-11a 3 g-2hg 8d-a 19d-a 7 g-4a 10e-b 13b-17f 18e-24 g C\]

The banding sequence ndt D2 was found only in one of the populations of the Eastern Caucasus (EC-AGR-CHT) with very low frequency (ndt D2 – 0.014) and only in the heterozygous state (ndt D1.2 – 0.029) (Table 3, 4).

**Arm E** was monomorphic with banding sequence ndt E1.1 (Fig. 2, Table 3, 4).

**Arm F** has two banding sequences: ndt F1 and ndt F2 (Figs. 2, 5). In most of the Caucasian populations (in seven from ten) the banding sequence ndt F2 was predominant, while other banding sequence ndt F1 was predominant in one of the populations of Central Caucasus (CC-ZAR-P) and both populations of Northwest Caucasus (Table 3, 4). In populations of Northwest Caucasus, the zygotic combination ndt F1.1 was predominant. The heterozygous combination ndt F1.2 was predominant in three populations of Central Caucasus (CC-NLC-P, CC-SKL-P and CC-KMS-L). The homozygous combination ndt F2.2 was predominant in both populations of Eastern Caucasus and one of the populations of Central Caucasus (CC-VG-LA).

**Arm G** has four banding sequences: ndt G1, ndt G2, ndt G1k and ndt G2k (Fig. 6, Table 3, 4).

In most of the Caucasian populations (in seven from ten) the banding sequence ndt G1 was predominant, while another sequence ndt G2 was predominant in one of the populations of Central Caucasus (CC-KMS-L) and both populations of Eastern Caucasus (Table 3, 4). We found the banding sequence ndt G1k only in one of the populations of Central Caucasus (CC-VG-LA). We found the other banding sequence ndt G2k only in the populations of Eastern Caucasus.

The zygotic combination ndt G1.1 was predominant in the populations of the Northwest Caucasus. The heterozygous combination ndt G1.2 was predominant in three populations of Central Caucasus (CC-ZAR-P, CC-GHN-LS and CC-KMS-L). The homozygous combination ndt G2.2 was predominant in both populations of Eastern Caucasus and one of the populations of Central Caucasus (CC-SKL-P). We found the zygotic combination ndt G1.1k only in one of the populations of Central Caucasus (CC-VG-LA) with pretty low frequency (0.056). The banding sequence ndt G2k probably is new for the species and described for the first time (Fig. 6, Table 3, 4). It has the same banding pattern as the sequence ndt G2 but with the DNA-knob at the telomeric end. The banding sequence ndt G2k was found only in the populations of the Eastern Caucasus with very low frequency (ndt G2k – 0.020, 0.057) and only in the heterozygous state (ndt G2.2k – 0.041, 0.029) (Table 3, 4).

### 3.3. Comparison of chromosomal polymorphism of Ch. nuditarsis from the Caucasian populations versus the populations of other regions

The data for European and Siberian populations are present due to Kiknadze et al. (2006).

**Arm A.** The arm in European populations was the most polymorphic in comparison with other populations and characterized by the presence of six banding sequences ndt A1, ndt A2, ndt A3, ndt A4 ndt A5 and ndt A6 (Table 3). At the same time, banding sequence ndt A1 and zygotic combination ndt A1.1 were predominant in these populations (Table 3, 4). Quite a similar picture was observed in the Caucasian populations, where we found five...
Table 3  
Frequency of banding sequences in different populations of Ch. nuditarsis. N – the number of individuals, ^ - data provided per Polukonova and Karmokov (2013), * - original data.

| Banding sequences | European population | Northwest Caucasian populations | Central Caucasian populations | East Caucasian populations | Siberian populations |
|-------------------|---------------------|---------------------------------|-------------------------------|--------------------------|---------------------|
|                   | EU-BE-WA            | EU-BG-CO                        | EU-IT-TU                     | NWC-PSB-FC              | NWC-MEZ-BP          |
|                   | N = 44              | N = 16                          | N = 26                        | N = 38*                  | N = 36*             |
|                   | A1                  | 0.591                           | 0.845                        | 0.826                    | 0.5                  |
|                   | A2                  | 0.250                           | 0.062                        | 0.154                    | 0.5                  |
|                   | A3                  | 0.023                           | 0.020                        | 0                        | 0.5                  |
|                   | A4                  | 0.102                           | 0.011                        | 0                        | 0.5                  |
|                   | A5                  | 0.023                           | 0.062                        | 0                        | 0.5                  |
|                   | A6                  | 0.011                           | 0.031                        | 0                        | 0.5                  |
|                   | A7                  | 0.023                           | 0.031                        | 0                        | 0.5                  |
|                   | B1                  | 0.795                           | 0.562                        | 0.808                    | 0.5                  |
|                   | B2                  | 0.205                           | 0.138                        | 0.192                    | 0.5                  |
|                   | C1                  | 1                               | 1.0                           | 0.962                    | 0.5                  |
|                   | C2                  | 0                               | 0.038                        | 0                        | 0.5                  |
|                   | D1                  | 1                               | 1.1                           | 1.1                      | 0.5                 |
|                   | E1                  | 0.989                           | 1.0                           | 1.1                      | 0.5                 |
|                   | E2                  | 0.011                           | 0.0                           | 0                        | 0.5                 |
|                   | F1                  | 1                               | 1.1                           | 0.737                    | 0.5                  |
|                   | F2                  | 0                               | 0.0                           | 0.263                    | 0.5                  |
|                   | G1                  | 0.693                           | 0.688                        | 0.885                    | 0.5                |
|                   | G2                  | 0.307                           | 0.250                        | 0.115                    | 0.5                |
|                   | G1k                 | 0                               | 0.062                        | 0                        | 0.5                |
|                   | G2k                 | 0                               | 0.0                           | 0                        | 0.5                |
| Heterozygous larvae, % | 81.8                | 87.5                            | 84.2                         | 91.6                    | 88                  |
| Average number of heterozygous inversions per larvae | 1.4                  | 1.4                            | 1.5                         | 1.25                    | 1.4                |
**Fig. 3.** Two different (a, b) photographs of heterozygous zygotic combination ndt A1.7. Designations as in Fig. 2.

**Table 4**

Frequency of zygotic combinations in different populations of *Ch. nuditarsis*. N – the number of individuals, ^ - data provided per Polukonova and Karmokov (2013), * - original data.

| Zygotic combinations                  | European populations | North-West Caucasian populations | Central Caucasian populations | East Caucasian populations | Siberian populations |
|---------------------------------------|----------------------|----------------------------------|--------------------------------|---------------------------|----------------------|
|                                       | EU- BE-              | EU- BG- CO-                      | EU- TU-                        | NWC- PSB- FC              | NWC- MEZ- BP          |
|                                       | WA N = 16            | N = 26                           | N = 38*                        | N = 36*                   | N = 38*               |
| A1.1                                  | 0.341               | 0.752                            | 0.654                          | 0.474                     | 0.278                |
| A1.2                                  | 0.045               | 0                                 | 0.052                          | 0.167                     | 0                    |
| A1.3                                  | 0.341               | 0.062                            | 0.338                          | 0.474                     | 0.389                |
| A1.4                                  | 0.091               | 0                                 | 0.083                          | 0                         | 0                    |
| A1.5                                  | 0                   | 0.062                            | 0                              | 0                         | 0                    |
| A1.6                                  | 0.045               | 0.062                            | 0                              | 0                         | 0                    |
| A1.7                                  | 0                   | 0                                 | 0                              | 0                         | 0                    |
| A2.1                                  | 0.068               | 0                                 | 0.083                          | 0                         | 0                    |
| A2.6                                  | 0                   | 0                                 | 0                              | 0                         | 0                    |
| A3.4                                  | 0.023               | 0                                 | 0                              | 0                         | 0                    |
| A3.5                                  | 0.023               | 0                                 | 0                              | 0                         | 0                    |
| B1.1                                  | 0.614               | 0.312                            | 0.263                          | 0.056                     | 0                    |
| B1.2                                  | 0.363               | 0.5                               | 0.536                          | 0.361                     | 0.583                |
| B2.2                                  | 0.023               | 0.188                            | 0.039                          | 0.184                     | 0.240                |
| C1.1                                  | 1                   | 1.923                            | 1                              | 1                         | 1                    |
| C1.2                                  | 0                   | 0.077                            | 0                              | 0                         | 0                    |
| D1.1                                  | 1                   | 1                                 | 1                              | 1                         | 1                    |
| D1.2                                  | 0                   | 0                                 | 0                              | 0                         | 0                    |
| E1.1                                  | 0.977               | 1                                | 1                              | 1                         | 1                    |
| E1.2                                  | 0.023               | 0                                  | 0                              | 0                         | 0                    |
| F1.1                                  | 1                   | 1                                 | 0.632                          | 0.417                     | 0.400                |
| F1.2                                  | 0                   | 0                                 | 0.211                          | 0.333                     | 0.400                |
| F2.2                                  | 0                   | 0                                 | 0.157                          | 0.250                     | 0.200                |
| G1.1                                  | 0.477               | 0.375                            | 0.77                           | 0.711                     | 0.360                |
| G1.1k                                 | 0                   | 0.125                             | 0                              | 0                         | 0                    |
| G1.2                                  | 0.432               | 0.5                               | 0.23                           | 0.289                     | 0.440                |
| G2.2                                  | 0.091               | 0                                 | 0                              | 0                         | 0.200               |
| G2.2k                                 | 0                   | 0                                 | 0                              | 0                         | 0                    |
Fig. 4. Heterozygous zygotic combination ndt D1.2. Designations as in Fig. 2.

Fig. 5. Homozygous zygotic combination ndt F2.2. Designations as in Fig. 2.

Fig. 6. Zygotic combinations in arm G: a – ndt G1.1, b – ndt G1.2, c – ndt G1.1k, d – ndt G2.2, e – ndt G2.2k. Designations as in Fig. 2.
banding sequences ndt A1, ndt A2, ndt A3, ndt A4 and ndt A7 (Table 3, 4). In most of the Caucasian populations as just as in European populations the banding sequence ndt A1 and zygotic combination ndt A1.1 were predominant. At the same time in Siberian populations the arm was monomorphic, only one banding sequence ndt A2 and one zygotic combination ndt A2.2 were present (Table 3, 4). These features clearly differed Siberian populations from other studied ones.

Arm B had two banding sequences ndt B1 and ndt B2 in European populations with predominance of the sequence ndt B1 (Table 3). Also, three zygotic combinations ndt B1.1, ndt B1.2 and ndt B2.2 with predominance of the combination ndt B1.1 were present in these populations (Table 4). Contrary in populations of Siberia only one banding sequence ndt B1 and one zygotic combination ndt B1.1 were present. The picture in this arm in some populations of Caucasus (mostly Central Caucasus) was similar to the picture in European populations and in the other populations (mostly Eastern Caucasus and a little less Northwest Caucasus) was similar to the picture in Siberian populations (Table 3, 4). In contrast to both European and Siberian populations; in four Caucasian populations, (NWC-PSB-FC, CC-ZAR-P, CC-SKL-P and CC-KMS-L) monomorphic combination ndt B1.2 was present.

Arm C has been monomorphic in all the Caucasian populations just as in most of European and Siberian populations, and characterized by the presence of banding sequence ndt C1 and zygotic combination ndt C1.1 (Table 3, 4). Only in one European population (EU-IT-TU) alternative sequence ndt C2 was present and found only in the heterozygous state.

Arm D has been monomorphic in most of Caucasian populations, just as in all the European and Siberian populations, and characterized by the presence of banding sequence ndt D1 and zygotic combination ndt D1.1 (Table 3, 4). Only in one population of the Caucasian (EC-AGR-CHT) alternative sequence ndt D2 was present and found only in the heterozygous state.

Arm E has been monomorphic in all the Caucasian populations, just as in most of European and Siberian populations, and characterized by the presence of banding sequence ndt E1 and zygotic combination ndt E1.1 (Table 3, 4). Only in one European population (EU-BE-WA) alternative sequence ndt E2 was present and found only in the heterozygous state.

Arm F of Ch. nuditarsis has been monomorphic in all the European and Siberian populations and characterized by the presence of banding sequence ndt F1 and zygotic combination ndt F1.1 (Table 3, 4). Contrary in the Caucasian populations the arm F was polymorphic. In the populations of Caucasus, alternative banding sequence ndt F2 was present in both homozygous and heterozygous state (Table 3, 4). These features are clearly differentiate Caucasian populations from all other studied ones. In the populations of Northwest Caucasus, the homozygous combination ndt F1.1 was predominant. In most of populations of Central and both the populations of Eastern Caucasus, the homozygous combination ndt F2.2 was predominant (Table 4). In a half of populations of Central Caucasus (CC-ZAR-P, CC-NLC-P, CC-SKL-P and CC-KMS-L), the frequency of the heterozygous combination ndt F1.2 reached quite high values (0.400–0.475) (Table 4).

Arm G is polymorphic in all the studied populations. In the European populations, two inversions banding sequences ndt G1, ndt G2 and one banding sequence with DNA-knob ndt G1k were present with predominance of zygotic combinations ndt G1.1. A similar picture - the presence of the inversion banding sequence ndt G1 and the sequence with DNA-knob ndt G1k, the predominance of zygotic combinations ndt G1.1 observed in the Siberian populations. The picture of polymorphism in Caucasian populations was slightly more complicated. In both populations of the Northwest Caucasus and two populations of the Central Caucasus (CC-NLC-P, CC-VG-LA) the homzygous combinations ndt G1.1 was predominant. The alternative homozygous combination ndt G2.2 was predominant in both populations of the Eastern Caucasus and one population of the Central Caucasus (CC-SKL-P). The heterozygotic zygotic combinations ndt G1.2 was predominant in three populations of the Central Caucasus (CC-ZAR-P, CC-GHN-LS and CC-KMS-L). In one population of the Central Caucasus (CC-VG-LA), the sequences with the DNA-knob ndt G1k was present in heterozygous state (ndt G1.1k) with a quite low frequency (0.056). In both populations of the Eastern Caucasus, other sequence with the DNA-knob ndt G2k was present also in the heterozygous state (ndt G2.2k) also with quite low frequencies (0.029, 0.041 respectively).

The level of inversion polymorphism of Caucasian Ch. nuditarsis populations is quite high (Table 3). The populations of the Caucasus are generally closer to European populations by the studied parameters of chromosomal polymorphism than to the populations of Siberia. As just in European, in populations of Caucasus the percentage of heterozygous larvae is quite high and varies from 50 to 91.6%. The average number of heterozygous inversions per larva also is quite similar to those of European populations (1.4) and varies in populations of Caucasus from 0.7 to 1.5 (Table 3).

We used the software package GenALEX 6.503 (Peakall and Smouse 2006, 2012) to check if the Caucasian populations follow Hardy-Weinberg expectation.

We performed a principal component analysis (PCA) of all the studied populations using original and previous data of Kiknadze et al. (2006) to obtain a broader overview of the population genetic relationships (Fig. 7).

We measured the genetic distances (Table 5) between populations by Nei criteria (1972) based on original and previous data of Kiknadze et al. (2006). Also, we constructed the tree dendrogram of genetic distances of the studied populations using single-linkage clustering based on the obtained values (Fig. 8).

4. Discussions

We found the species Ch. nuditarsis in the Northwest and East Caucasus for the first time. Earlier Polukonova and Karmokov (2013) recorded the species for the Central Caucasus. All our data are indicative that in Caucasus region, we didn’t deal with form II of Michaelova (1989).

The Caucasian populations of Ch. nuditarsis can be overall, characterized as highly polymorphic. We observed inversion polymorphism in almost all chromosome arms except for arm C and E, which were monomorphic in Caucasian populations.

On the dendrogram of genetic distances, there are four clear clusters that we conditionally assigned as European, Central Caucasian, East Caucasian and Siberian clusters (Fig. 8). In spite of relative geographic proximity, the genetic distances between some Caucasian populations are quite large (Table 5), and they do not form a single cluster of Caucasian populations. The European cluster formed by the populations of the Europe and Northwest Caucasus. The Central Caucasian, East Caucasian and Siberian populations form their own separate clusters.

In general, the genetic distances between populations within the same clusters (Table 5; Fig. 8) do not reach the distance range (0.136 ± 0.026) for different population of the one species (Gunderina, 2001). One can consider them as a one big population. For example, the distance between populations of Siberian cluster is 0.002. Almost the same picture with the low distance (0.046) between populations we observed in an East Caucasian cluster (Table 5). The distances between most of populations of Central Caucasian cluster (0.020–0.100) also do not reach the distance range (0.136 ± 0.026) for different population of the one species (Gunderina, 2001). Just in European cluster (Table 5) one popula-
tion of the Northwest Caucasus (NWC-MEZ-BP) separated from other populations at the level of different population of the one species (the distance between populations EU-IT-TU and NWC-MEZ-BP is 0.141) or even at the level (0.474 ± 0.314) of subspecies (Gunderina, 2001). The distances between populations EU-BE-WA, EU-BG-CO and NWC-MEZ-BP are 0.169 and 0.182 respectively.

At the same time, the distances between populations from different clusters are quite big and even reach a level of subspecies (0.474 ± 0.314) (Gunderina, 2001). The distances between populations of East Caucasian cluster and the populations of all other clusters are quite big and varies from 0.248 to 0.555 (Table 5). The distances between populations of Siberian cluster and the populations of all other clusters even bigger and varies from 0.400 to 0.643 (Table 5).

The principal component analysis shows almost the same picture as the dendrogram of genetic distances (Fig. 7). One can see the same four clearly separated clusters. The populations of the European cluster are characterized by a constant increase of frequencies of zygotic combinations ndt F1.1 and ndt G1.1. The Central Caucasian cluster is characterized by increasing frequencies of other combinations ndt A1.1 and ndt F1.2. The East Caucasian cluster is characterized by increasing frequencies of combinations ndt G2.2 and ndt F2.2. The Siberian cluster is characterized by high frequencies of zygotic combination ndt A2.2. The European and Central Caucasian clusters are closest to each other.

We suppose that there are several ways to explain the observed picture. On one hand, such a clear separation of Caucasian populations in distinct clusters could be a result of differences of collection sites in terms of geography and climate. Due to complex, diverse terrain and climate conditions in the Caucasus region, it is possible that in every separate collection site there are unique set of environmental parameters. On other hand, the observed picture could be explained as insufficiency of our data. Probably, if we will add to analysis more data on more populations from other regions of Caucasus the gaps between clusters will be filled and we will see the gradual transition of populations of the species from Northwest to East Caucasus.

The frequencies of zygotic combinations in chromosome arms of *Ch. nuditarsis* in the most part of the Caucasian populations (six of ten) follow Hardy-Weinberg expectation. In four Caucasian populations, mostly in the arms B, F and G, the frequencies of zygotic combinations do not follow Hardy-Weinberg expectation.

### Table 5
Values of genetic distances between the different populations of *Ch. nuditarsis*.

| Populations       | EU-BE-WA | EU-BG-CO | EU-IT-TU | NWC-PSB-FC | NWC-MEZ-BP | CC-ZAR-P | CC-NLC-P | CC-SKL-P | CC-GHN-L | CC-KMS-L | CC-VG-LA | EC-SAM-CR | EC-AGR-HT | SB-NSK-KR | SB-RAL-Te |
|-------------------|----------|----------|----------|------------|------------|----------|----------|----------|----------|----------|----------|-----------|-----------|-----------|-----------|
| EU-BE-WA          | 0        |          |          |            |            |          |          |          |          |          |          |           |           |           |           |
| EU-BG-CO          | 0.043    | 0        |          |            |            |          |          |          |          |          |          |           |           |           |           |
| EU-IT-TU          | 0.024    | 0.046    | 0        |            |            |          |          |          |          |          |          |           |           |           |           |
| NWC-PSB-FC        | 0.054    | 0.064    | 0.044    | 0          |            |          |          |          |          |          |          |           |           |           |           |
| NWC-MEZ-BP        | 0.169    | 0.182    | 0.141    | 0.053      | 0          |          |          |          |          |          |          |           |           |           |           |
| CC-ZAR-P          | 0.148    | 0.070    | 0.131    | 0.082      | 0.115      | 0        |          |          |          |          |          |           |           |           |           |
| CC-NLC-P          | 0.206    | 0.181    | 0.158    | 0.130      | 0.161      | 0.065    | 0        |          |          |          |          |           |           |           |           |
| CC-SKL-P          | 0.273    | 0.224    | 0.274    | 0.238      | 0.337      | 0.100    | 0.128    | 0        |          |          |          |           |           |           |           |
| CC-GHN-L          | 0.200    | 0.163    | 0.198    | 0.167      | 0.279      | 0.065    | 0.044    | 0.144    | 0        |          |          |           |           |           |           |
| CC-KMS-L          | 0.192    | 0.152    | 0.192    | 0.125      | 0.211      | 0.032    | 0.039    | 0.079    | 0.020    | 0        |          |           |           |           |           |
| CC-VG-LA          | 0.242    | 0.230    | 0.210    | 0.204      | 0.281      | 0.130    | 0.037    | 0.209    | 0.025    | 0.065    | 0        |           |           |           |           |
| EC-SAM-CR         | 0.403    | 0.287    | 0.396    | 0.287      | 0.248      | 0.127    | 0.158    | 0.135    | 0.184    | 0.131    | 0.241    | 0         |           |           |           |
| EC-AGR-HT         | 0.555    | 0.442    | 0.542    | 0.426      | 0.362      | 0.258    | 0.248    | 0.179    | 0.276    | 0.228    | 0.293    | 0.046     | 0         |           |           |
| SB-NSK-KR         | 0.280    | 0.287    | 0.279    | 0.251      | 0.152      | 0.400    | 0.456    | 0.628    | 0.610    | 0.550    | 0.610    | 0.425     | 0.540     | 0         |           |
| SB-RAL-Te         | 0.282    | 0.295    | 0.275    | 0.248      | 0.144      | 0.405    | 0.451    | 0.643    | 0.616    | 0.557    | 0.610    | 0.439     | 0.555     | 0.002     | 0         |

Fig. 7. Principal component analysis (PCA) of zygotic combination frequencies in 15 *Ch. nuditarsis* populations: diamonds – European populations, squares – Northwest Caucasian populations, dots – Central Caucasian populations, triangles – Siberian populations, inv. triangles – East Caucasian populations.
Weinberg expectation ($\chi^2 = 7.941, p = 0.005$). The heterozygotes ndt F1.2 should occur 2.21 times as frequently as observed and homozygotes ndt F2.2 observed 2.28 times as frequently as expected. One can observe more complex picture in population CC-NLC-P from Central Caucasus, where the frequencies of zygotic combinations do not follow Hardy-Weinberg expectation across two arms: arm B ($\chi^2 = 5.766, p = 0.016$) and arm G ($\chi^2 = 9.095, p = 0.003$). In the arm B the heterozygotes ndt B1.2 should occur 1.93 times as frequently as observed and the homozygotes ndt B2.2 observed 2.39 times as frequently as expected. In the arm G the heterozygotes ndt G1.2 should occur 2.525 times as frequently as observed and the homozygotes ndt B2.2 observed 2.56 times as frequently as expected. In another one population from the Central Caucasus (CC-GHN-L) the frequencies of zygotic combinations in arm G do not follow Hardy-Weinberg expectation ($\chi^2 = 5.0620, p = 0.024$). The heterozygotes ndt G1.2 observed 1.3 times as frequently as expected and homozygotes ndt G1.1 should occur 1.32 times as frequently as observed. In one of two populations from East Caucasus (EC-SAM-CR) the frequencies of zygotic combinations in arm B do not follow Hardy-Weinberg expectation ($\chi^2 = 59.599, p = 0.000$). The heterozygotes ndt B1.2 observed 1.9 times as frequently as expected, the homozygotes ndt B2.2 should occur 5.15 times as frequently as observed. The expected homozygotes ndt B1.1 that should occur with notable frequency (0.224) was not found at all.

One can suggest that in first two populations (NWC-PSB-FC, CC-NLC-P) in arms B, F and G there is probably a negative selection of heterozygotes B1.2, F1.2, G1.2 present. Contrarily, in other two populations (CC-GHN-L, EC-SAM-CR) in arms B and G there is probably a positive selection of heterozygotes B1.2, G1.2 present. Apparently, such a selection process is more expressed in population EC-SAM-CR in the arm B where one can observe the highest $\chi^2$ value and lowest probability. Concerning the picture in population EC-SAM-CR there is one other possible explanation. We suppose that in differ from other three collection sites in this population due to warmer climate conditions more than one generation during the summer is possible. This means that the samples could be different generations and so have been subject to different selection pressures.

We suggest that observed possible multi-directional selection of heterozygotes in different populations could be a reflection of some adaptive processes occurring in these populations.

5. Conclusions

All the obtained data are indicative of the complex genetic structure of Caucasian populations of Ch. nuditarsis and total complexity of microevolution processes occurring in the Caucasus region. In spite of geographic proximity, Caucasian populations do not form a single cluster of Caucasian populations, probably due to differences of collection sites in terms of geography and climate (complex, diverse terrain and microclimate conditions). The populations of Ch. nuditarsis from different parts of the Caucasus diverged from each other at the level of subspecies. In the different populations, one can observe possible adaptive processes.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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