The last Pleistocene glaciations phylogeography episode of *Phaneroptera falcata* (Poda, 1761) (Orthoptera: Tettigoniidae) in the Volga River basin based on the mtDNA Cytochrome C Oxidase subunit 1 (COI) gene fragment

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Abstract

This study is to research the phylogeography of *Phaneroptera falcata* (Poda, 1761) in the Volga river basin based on the mtDNA Cytochrome C Oxidase subunit 1 (COI) gene fragment at the last Pleistocene glaciation episode. The studied location is the Volga river basin, a territory within the central and partially southern parts of European Russia; it includes the rivers Volga, Oka, Khoper and Don basins. We used the traditional molecular phylogeography methods: mtDNA COI gene fragment from the key locations within the studied area was sequenced and then analyzed (cladogram topology, haplotype di-
versity, cladogram calibration etc.). The phylogenetic tree shows the dispersion of our samples over the following regions: Lower Volga, Middle Volga, Lower Oka, Middle Oka, Upper Oka, Don basin, Khoper basin. Nine haplotypes determined from our samples; they are grouped into 7 haplogroups. Six of them are in the basins of the main rivers of the Volga region: three haplogroups - on the Oka (Upper, Middle and Lower Oka respectively), haplogroups of the Khoper and Don basins, and the haplogroup of the Middle Volga combining two subgroups – Lower and Middle Volga basins. The distribution of found haplogroups correlates with big river basins in the Volga area (Volga, Oka, Khoper, Don).

Keywords
Orthopteroid insects, distribution, phylogeography, dispersion age

Introduction
Phaneropterinae is one of the largest subfamilies in the family Tettigoniidae. There are 338 valid genera and 2162 species in the world fauna (Cigliano et al. 2020). Phaneroptera Audinet Serville, 1831 is distributed in Eurasia and Africa (Bey-Bienko 1954; Storozhenko 2004). It has about 38 valid species (Storozhenko and Paik 2007; Cigliano et al. 2020), two of them are found in Russia. Most of the species of this genus occur in tropical areas. Phaneroptera falcata (Poda, 1761) (figs 1, 2) is a trans-eurasian subboreal species (Sergeev 1986). It occurs in southern and central parts of European Russia as well as in South Siberia, Transbaikalia, South of the Amur Region, Khabarovsk Krai and Primorsky Krai. It also inhabits Europe outside of Russia, Northern Iran, Caucasus, Transcaucasia, Kazakhstan, Central Asia, Mongolia, China, Taiwan, Korea, and Japan (Storozhenko 2004; Storozhenko and Paik 2007). Taking into account the latest data on the distribution of P. falcata, the species belongs to the forest-steppe, Palaearctic complex of species. The species inhabits meadows, forest margins and closely related biotopes (figs 3, 4). It is meso-xerophilic, often found in pastures and wastelands, but requires stand canopy density and a certain height of grass (Adakhovsky 2006). It prefers lowland (flood) meadows with sedges, open areas with shrubs and grasses, and margins of floodplain forests (on the border of the ecotone).

P. falcata feeds on the trees and bushes as far as on the tall grass species, its food preferences are young leaves and juicy fruits. Population fluctuations of P. falcata can be economically dangerous at this time because it can damage some crops.

According to current research, recently in Eurasia, the range of P. falcata has expanded in the western, north-western and eastern directions (Felix et al. 2004; Kočárek and Holuša 2006; Grein, 2007; Iorgu et al. 2008; Ivinskis and Rimšaitė 2008; Kočárek et al. 2008; Zuna-Kratky et al. 2009; Böhme et al. 2011; Handke et al. 2011; Stohr et al. 2011; Sokolovskis and Suveizda 2012; Budrys et al. 2015; Aleksandrowicz 2017). In Russia during last 25 years, P. falcata has spread to the east, reaching the Tyumen (Sergeeva and Kapitonov 2017) and Novosibirsk regions (Sergeev 2004). Since 2003, P. falcata has been regularly found not only in the south, but also in the north-east of the Trans-Ural forest zone (Stolbov et al. 2016). In West Siberia, P. falcata has
been recorded in the south of the Omsk region and in the Altai territory (Efremova and Sergeev 2009). In addition, Adakhovsky (2016), Ozersky and Tislenko (2015), Ozersky (2017, 2019) Ruchin and Mikhailenko (2018) noted its active distribution northwards and westwards. The species was discovered in the Novgorod region in 2013 and in Saint Petersburg in 2015 (Ozersky and Tislenko 2015). In 2011, *P. falcata* was recorded in Kirov and in the South-East of the Kirov region (Yuferev 2011).

Area fluctuation is a natural phenomenon in zoogeography. Today it is common, as global warming causes thermophilic species to colonise the new areas that have become habitable (Berezenko and Milchakova 2018; Mikhaylov and Vinarski 2018;

![Figures 1–4. Phaneroptera falcata in nature and its biotope. 1, 2: insect in nature; 1 – Tula Region, Belgorodye village environs, photo by A. Mikhailenko; 2 – Volzhsko-Kamsky Nature Reserve, Raif part, photo by N. Shulaev. 3, 4: biotopes; 3 – Tararstan Republic, Belaya river valley, photo by N. Shulaev; 4 – Republic of Mordovia, near Temnikov, photo by A. Ruchin.](image-url)
During the Quarternary period, there were many climate fluctuations that affected the composition of flora and fauna in different historical periods (Elias 2013; Kurnaz and Kutrup 2019). Using modern methods, in particular, the analysis of DNA markers, it is possible to find out the main ways of forming the modern range of a particular species with a high degree of credibility (Todisco et al. 2010; Kaya and Çiplak 2017; Wang et al. 2019). In similar phylogeographic studies, orthopteran insects were model objects (Bugrov et al. 2010; Ferreira and Ferguson 2010; Kindler et al. 2012; Kaya et al. 2015; Chobanov et al. 2016; Mugleston et al. 2018; Çiplak et al. 2020).

Such a study has never been conducted for *P. falcata*. In this paper, based on the analysis of the mtDNA COI sequence, we study the phylogeography of *P. falcata* in the Volga basin.

**Material and methods**

DNA testing was performed on 16 samples (Table 1). The first Cytochrome Oxidase subunit (COI) was selected for testing. Sequencing was performed at the University of Guelph (Canada) as part of the BOLD project (Ratnasingham and Hebert 2007) using protocols described by Wilson (2012).

The material was collected in the field by entomological netting. After collection, the material was placed into 95% ethanol to store it. Data processing was performed using the following software: Microsoft Excel, SPSS Statistica for Windows, MEGA X, BioEdit version 7.2.5, DNASP version 1.2.5, BEAST ver. 2.0.

The sequence KY963195 (*Phaneroptera nigroantennata* Brunner von Wattenwyl, 1878) mined from the GenBank database used as an outgroup for phylogenetic reconstructions. In addition, the following sequences from the BOLD database were used: GBMH3924-08 (Spain, Barcelona; *P. falcata*); OTAS353-14 (Australia, New South Wales; undefined species); RBTC1711-16 (China, Zheyang, *P. nigroantennata*); RBTC1820-16 (China, Zheyang, undefined species); RBTC1905-16 (China, Zheyang, undefined species); RBTC593-16 (China, Hebei, undefined species); RBTC599-16 (China, Ganxi, undefined species).

**Table 1.** Material sequenced in this study.

<table>
<thead>
<tr>
<th>№</th>
<th>Sequence ID</th>
<th>Collection date</th>
<th>Region</th>
<th>Locality</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PF27</td>
<td>-</td>
<td>Kaluga Region</td>
<td>Yuhchnov Distr., Pavlistshev Bor</td>
</tr>
<tr>
<td>2</td>
<td>PF28</td>
<td>23.09.2006</td>
<td>Moscow Region</td>
<td>Serpukhov distr., southern borders of Prioksko-Terrasny Nature Reserve</td>
</tr>
<tr>
<td>3</td>
<td>PF29</td>
<td>31.07.2007</td>
<td>Republic of Tatarstan</td>
<td>Nizhniiyaya Kama</td>
</tr>
<tr>
<td>4</td>
<td>PF31</td>
<td>08.2008</td>
<td>Republic of Tatarstan</td>
<td>Alkeevsky Distr., Chubvrod forest farm</td>
</tr>
<tr>
<td>5</td>
<td>PF32</td>
<td>02.07.2015</td>
<td>Penza Region</td>
<td>Serdobsk Distr., NE of Konstantinovka</td>
</tr>
<tr>
<td>6</td>
<td>PF33</td>
<td>20.07.2016</td>
<td>Samara Region</td>
<td>Zhigulevsky Nature Reserve, Bakhchilova Polyana</td>
</tr>
</tbody>
</table>
Results

The phylogenetic tree (Fig. 5) shows the dispersion of our samples over the following regions: Ulyanovsk and Samara Regions (the Lower Volga), Tatarstan and the southeast of Chuvashia (the Middle Volga), the Republic of Mordovia and the south-west of Chuvashia (the Lower Oka, Lower Sura), Moscow Region (the Middle Oka), Tula and Kaluga Regions (the Upper Oka), Lipetsk Region (the Don basin), Penza Region (the Khoper basin).

The geographical distribution of the analyzed sequences by the specified regions is shown in Fig. 6.

Nine haplotypes determined from our 16 samples (Fig. 7), the haplotype diversity is 0.927, the haplotype diversity variance is 0.001, the standard deviation is 0.038; haplotypes form seven haplogroups.

There are the following features for the distribution of haplotypes: the average value of nucleotide differences is 2.8 (the average p-distance is 0.02), 15 of the 658 bp are polymorphic, and the remaining sites are monomorphic. The region from 63 to 190 sites is identical for all studied sequences (the stored sequence), this is:

\[\text{TACCTAATTGGAGATGATCAAATTTATAATGTAATTGTTACTGCTCACGCATTGTGTAATAATCTTTATATGTTATACCTATTAATTGGAAGGATTTGGGATTTGATTAGTTCTTATATAATCTAGG.}\]

When analyzing the use of codons, the value of the scaled Chi-square ranges from 0.375 (the Khoper basin) to 0.416 (the Upper Oka); the average of the calibrated Chi-square is 0.380.

<table>
<thead>
<tr>
<th>№</th>
<th>Sequence ID</th>
<th>Collection date</th>
<th>Region</th>
<th>Locality</th>
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<tr>
<td>7</td>
<td>PF34</td>
<td>04.08.2018</td>
<td>Republic of Mordovia</td>
<td>Kochkourovo Distr., Podlesnaya Tavla</td>
</tr>
<tr>
<td>8</td>
<td>PF48</td>
<td>12.08.2018</td>
<td>Lipetsk Region</td>
<td>Lipetsk</td>
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<tr>
<td>9</td>
<td>PF49</td>
<td>14.08.2018</td>
<td>Republic of Mordovia</td>
<td>Insar Distr., Verkhnyaya Lukhma</td>
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<tr>
<td>10</td>
<td>PF51</td>
<td>14.08.2018</td>
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<td>Insar Distr., Verkhnyaya Lukhma</td>
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<tr>
<td>11</td>
<td>PF57</td>
<td>15.08.2018</td>
<td>Ulyanovsk Region</td>
<td>Inza Distr., Tiyapino</td>
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<tr>
<td>12</td>
<td>PF60</td>
<td>17.08.2018</td>
<td>Chuvash Republic</td>
<td>Yalchiksky Distr., Eshmikeovo, Yalchiksky cluster of the Nature Reserve «Prisursky»</td>
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<td>13</td>
<td>PF66</td>
<td>18.08.2018</td>
<td>Moscow Region</td>
<td>Serebryanyi-Prud Distr., SW of Krutovez</td>
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<tr>
<td>14</td>
<td>PF70</td>
<td>29.08.2018</td>
<td>Tula Region</td>
<td>Vodyanoe Pole</td>
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<tr>
<td>15</td>
<td>PF76</td>
<td>30.08.2018</td>
<td>Chuvash Republic</td>
<td>Alatyrsky Distr., Atrat, Alatyrsky cluster of the Nature Reserve «Prisursky»</td>
</tr>
<tr>
<td>16</td>
<td>PF80</td>
<td>2018</td>
<td>Republic of Tatarstan</td>
<td>Zelenodolsk Distr., Volga-Kama Nature Reserve</td>
</tr>
</tbody>
</table>
The selected haplotypes are grouped into seven haplogroups. Six of them are in the basins of the main rivers of the Volga region: three haplogroups - on the Oka (the Upper, the Middle and the Lower Oka respectively), haplogroups of the Khoper and Don basins, and the haplogroup of the Middle Volga combining two subgroups – the Lower and the Middle Volga basin, and the Middle Volga.

**Figure 5.** Phylogenetic tree of *Phaneroptera falcata* of the Volga region. ML, Kimura-2 parameter model, 10000 bootstrap-replications.

**Discussion**

Pleistocene glaciations were located along the beds of large rivers far to the south. According to Sibrava et al. (1986), during the glacial maxima of the early Valdai glaciation (Shestikhinsky and upper Volga maxima), the placore glaciation along riverbeds reached the Lower Don, Khoper and Oka. *P. falcata* has features of distribution in the Volga region: the main haplogroups are strictly confined to the basins of large rivers, which recently were the “guides” of the ice sheets.

Based on the distribution of haplotypes of *P. falcata* it is possible to recover partially the ways of the migrations of its ancestor in its European part of area (in the Volga river basin).

The phylogeography of *P. falcata* in the last glaciation period shown in Fig. 8. It has three main events received from the Bayesian analysis of our sequences set. 1st divergence of the ancestral population to South European and Central European branches (Volga river basin populations belong to the Central European branch
and marked in Fig. 8 by grey color). 2nd divergence of Khoper and Volga-Oka basins populations. 3rd divergence of Oka basin populations (upper course and middle course parts). The time calibration of these events is impossible due to the absence of fossils; we can, however, use the well-known geological history of this region to make approximate timings of these events.

There are the following stages in the history of distribution of the ancestor of *P. falcata* on the territory of the Volga river basin at the last glaciation time:

1. The ancestor of *P. falcata* colonised the southern parts of its modern range in European Russia and actively moved northwards. This is due to the gradual formation of conditions close to current in the pre-glaciation period of the middle Pleistocene (Elias 2013), which contributed to the adaptive radiation of forms close to modern. At that time, the most ancient haplogroups were formed: Oka, Volga, Khoper, Don.
2. The clades of the ancestor of *P. falcata* that inhabit the Oka and Volga basins were separated in the end of the Pleistocene period. About 10–15 000 years ago in the territory of the Oka river basin formed the system of rivers and their valleys close to its modern view (Runkov and Maskaikin 2018).

3. The division of haplogroups of the ancestor of *P. falcata* in the Ciscaucasus and the southern part of Western Europe. We cannot explain it in this study due to the lack of material, but the most likely reason for this division was the division of ancient ancestor populations into two large areas during the maximum of the Don glaciation.

4. Finally, the last event that can be traced is the separation of haplogroups of the Oka basin (the Lower and Upper Oka) after the last glaciation at the end of the Pleistocene period. At that time, the parts of the Moscow glaciation that divided the area of the ancestor of *P. falcata* into two sections (Upper and Lower ones) descended in narrow parts along this territory (Milanovsky 1940; Spiridonov 1954; Shik 2010).

So, basing on the current material, we can confirm the division of this subboreal hygromesophytous species according to the main river basins, where it forms the separate subpopulations characterized by their own haplotypes.

**Figure 7.** Haplotype network of sequenced *Phaneroptera falcata* samples. MJ.
Conclusions

Phylogeography of *P. falcata*, hygromesophytous species, colonize the basins of major rivers. The history of the group in the Volga basin, traced on the available material, correlates with the past geological events (last Pleistocene glaciation events); the largest of them are account for the key moments of settlement of the ancestor of this species in the region.

It is obvious that the model we have constructed for the distribution of the ancestor of *P. falcata* in the region will be similar for many other species with similar ecology; this can be verified using mtDNA markers.

It is necessary to study in detail the relationship between the populations of the ancestor of *P. falcata* of the Middle Volga region in the Quaternary period, as a region with the least studied Quaternary history.

![Figure 8](image_url)  
**Figure 8.** Phylogeography events of *Phaneroptera* in the Volga river basin in Pleistocene. Gray indicates the ancestor of *P. falcata* in the Volga river basin.

Acknowledgement

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References


Phylogeography of *Phaneroptera falcata*


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