**Original Article**

# Genetic Structure of *Aedes* (*Stegomyia*) *albopictus* Populations in Russia

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## Abstract

**Background:** *Aedes* (*Stegomyia*) *albopictus* was found for the first time in 2011 on the Black Sea coast in Russia, and during 2011–2019, the species expanded over two climate zones Cfa and Csa.

**Methods:** Here, we studied the sequence diversity of the mitochondrial cytochrome c oxidase I (*COI*) gene, 1317–1433bp in length. In total, 131 specimens of *Ae. albopictus* sampled from 21 locations in Russia and Abkhazia were examined.

**Results:** Two of the six identified mitochondrial haplotypes were detected for the first time. Four *COI* haplotypes were shared by at least two studied local populations. The most prevalent H1 and H2 haplotypes dominated in all the sampled localities in the Cfa zone. The H3 haplotype was prevalent in the Csa zone. Other haplotypes were rare. Phylogenetic analyses, spatial isolation and limited gene flow revealed that the samples from the Csa zone differed significantly from those from the Cfa zone.

**Conclusion:** Two spatially isolated genetic lineages exist in *Ae. albopictus* population in southern region of Russia. One lineage obtained on the seacoast and inland (in valleys of the Caucasus Mountains and steppe zone) is widely distributed worldwide including Mediterranean populations. This confirms the hypothesis that the emergence of *Ae. albopictus* population in southern region of Russia may be associated with the terrestrial spread of mosquitoes from the well-established European population due to human activity. The other lineage, discovered in Novorossiysk, a maritime port, is similar to *Ae. albopictus* from the USA and Japan, suggesting the independent introduction of these mosquitoes.

**Keywords:** *Aedes albopictus*; Distribution; Population genetics; *COI*; mtDNA

## Introduction

*Aedes* (*Stegomyia*) *albopictus* (Skuse, 1895) is recognized as a competent vector of arboviruses, including dengue, chikungunya, Zika and yellow fever viruses (1). Being initially native to restricted tropical regions of Southeast Asia, *Ae. albopictus* spread widely during the last 30 years in tropical and subtropical climates owing to its ecological features and human activity (2). In Europe, established populations of *Ae. albopictus* were recorded for the first time in Albania (1979) and Italy (1991), but currently, the species is reported to occur in countries of the Mediterranean basin, Balkans and central Europe, including the Czech Republic, and Germany (3–5). *Aedes albopictus* introduction and expansion led to epidemics of chikungunya fever in Italy and France and to autochthonous cases of dengue in France, Croatia and Spain (6–9).

On the Black Sea coast *Ae. albopictus* was first observed in 2011 in Bulgaria, Turkey, Georgia, Abkhazia and Russia, and the following year was found in Romania (4, 10–12). In Russia, it was originally recorded in only one location, Adler, near the Abkhazia border, but in subsequent years, *Ae. albopictus* spread along the coast, being discovered in 2015 in Gelendzhik and in 2016 in Novorossiysk (13–14). Monitoring of the species range showed that during the period 2011–2019, *Ae. albopictus* expanded along the coast for 400km and inland for 200km, establishing breeding populations in valleys of the Caucasus Mountains and in the steppe zone of the Krasnodar krai (15).

The widespread distribution of *Ae. albopic-*



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*tus* in southern Russia enhances the risk of autochthonous transmission of dengue, chikungunya and Zika viruses in a large area on the Black Sea coast, especially in summer, when many tourists from different countries visit the region (16). Such scenarios of disease outbreak occurred in Europe and in the USA (Florida) (6). The epidemic potential of *Ae. albopictus* depends on the mosquito population's vector competence and vectorial capacity, which are thought to vary in invading populations according to their genetic background and geographic origin (17, 18). Hence, information on the genetic diversity of invasive populations is important for survey of *Ae. albopictus* populations and disease risk assessment.

Currently, both nuclear and mitochondrial markers are used to analyse the genetic structure and origin of invasive *Ae. albopictus* populations (19). The application of a nuclear marker, the ITS2 region of rRNA genes, displayed a low level of differentiation between geographical populations, indicating that highly related sequences were distributed across native and invasive populations (19, 20). Analyses of microsatellites and a panel of genome-wide distributed Single Nucleotide Polymorphisms (SNPs) showed that low levels of intra-population differentiation were caused by multiple re-introductions into invaded zones, which led to the formation of local populations consisting of individuals with different origins (21–23). In Europe, a high admixture and lack of geographical structure was found based on ITS2 and SNPs (20, 22). This result suggests a long-distance human-aided dispersal of *Ae. albopictus* in Europe (22).

Mitochondrial DNA genes are often used as genetic markers to test biodiversity, ancestry and demographic changes in populations due to their uniparental (maternal) inheritance and lack of recombination (24). The cytochrome c oxidase I (*COI*) gene in *Ae. albopictus* has been widely analysed not only for species identification as a BARCODE tool but also through phylogenetic and population studies (25–29). A short BARCODE fragment of 450–650bp was used to confirm discovery of *Ae. albopictus* in Turkey (10), countries of the Balkan Peninsula (4, 30, 31). The limited sequence length and number of individuals analysed did not reveal a high level of diversity. At the same time, it is shown that the long fragment (circa 1400bp) of *COI* gene can be used to analyse the population structure of *Ae. albopictus*. A high level of polymorphism was detected among long *COI* sequences of *Ae. albopictus* sampled in native areas in South Asian countries (25, 26, 32–34). More than a thousand published *Ae. albopictus COI* sequences reflects the genetic diversity of populations both in native and non-native invasive areas, highlighting the reliability of the long *COI* fragment to identify genetic divergence among *Ae. albopictus* populations and facilitating the study of phylogeographic relationships and the probable origin of individuals in recently established populations (25, 27–29, 34).

Our aim was to study the genetic diversity and population structure of *Ae. albopictus* in the Krasnodar krai, Russia, and in Abkhazia. Two hypotheses regarding the structure of *Ae. albopictus* populations can be assumed. The simultaneous occurrence of the vector in the eastern Black Sea countries suggests a common single source of origin, from which the mosquitoes spread locally by different modes of transport. In this case, the population genetic diversity should be low, because the population has existed for less than 10 years, since 2011 (12). On the other hand, the possibility of independent importation by sea cannot be excluded, as both Russia and Abkhazia have ports on the Black Sea and then subpopulations and high genetic diversity can be expected due to multiple importations and mixing of individuals in local populations. To test these assumptions, we sampled mosquitoes in Krasnodar krai, Russia, and Abkhazia in 2018 and sequenced a long fragment of the *COI* gene. We compared *Ae. albopictus COI* haplotype composition between local populations across the studied area and with the haplotypes known for other endemic and non-endemic territories.


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## Materials and Methods

### Study area

Our study sites covered an area from 45° 13'N to 43°10' N and from 38°59'E to 40°20' E (Fig. 1, Table 1). According to the Köppen-Geiger classification, most of this area lies in the Cfa climate zone, defined as temperate (C) climate without dry season (f) and with a warm summer (a) (35). The Caucasus Mountains cross the Cfa zone and form a watershed that divides the zone into three climate subzones. The climate of seacoast of Abkhazia and the part of seacoast of Russia is close to subtropical climate with an average January temperature of > 6 °C and annual rainfall above 1400mm and denoted as the subzone Cfa-1 (Table 1). The valleys located on the western and northern slopes of the mountains are characterized by a mild temperate climate (Cfa-2) with an average January temperature of > 2.0 °C and annual rainfall of approximately 1000mm. The climate of the Zakubanskaya Plain located to the north and northwest of the Caucasus Mountains is mild continental (Cfa-3), with average January temperatures of > 0.0 °C and approximately 600–700mm of precipitation per year. To the west of the Caucasus Mountains, in Novorossiysk and its surroundings, the climate is close to the dry Mediterranean type (Csa), with precipitation of the driest month in summer < 40mm.

### Mosquito sampling

Mosquitoes were sampled at 17 sites in Krasnodar krai, Russia, and three sites in Abkhazia in 2018 (Fig. 1). The collection points were at a sufficient distance to avoid collecting siblings. The sampling sites were combined into seven groups on the basis of territory (Table 1). The collections were performed in August-September, when the population density of mosquitoes was expected to be the highest (36). Adult females were collected with aspirators and the human landing catch approach. For the collection of mosquito larvae, we inspected containers with water in cemeteries, used tires and plastic containers in yards or along roadsides, buckets and other types of containers. Mosquito larvae and pupae were harvested, brought to a lab, reared to adults and identified by morphological identification keys (37).

### DNA extraction, amplification, and sequencing

Each mosquito was homogenized in 300µl of saline buffer. The homogenate was centrifuged at 800g for 2min, and the supernatant was retained. Total DNA was extracted from 100µl of supernatant using the RiboPrep kit (Amplisense, Moscow, Russia) according to the manufacturer's instructions. PCR was carried out in a 25µl volume with an Encyclo Plus PCR kit (Evrogene, Moscow, Russia). The *COI* gene fragments were amplified using primers 1454F and 2160R and 2027F and 2886R according to the original protocol (25). The amplification products were sequenced with forward and reverse primers for each primers pair. Sequencing of amplicons was performed using an ABI PRISM 310 sequencer and a BigDye Termination kit as recommended by Applied Biosystems (United States). The sequences were edited manually with Chromas and then aligned using MAFFT v7 (https://mafft.cbrc.jp). The total length of the *COI* gene sequences was 1317–1433bp. The nucleotide sequences were deposited in GenBank under accession numbers MZ 501500-MZ501561. Sequences MH817490–MH817558 from our preliminary study (38) were also included in the analysis. A total 131 sequences from individual mosquitoes were used for the genetic analysis.

### Analysis of mtDNA data

The length of the aligned analysed sequences was 1317bp. DNA polymorphism was evaluated using DnaSP v5 (39). DnaSP software was used also to evaluate the values of number of migrants (Nm), level of population differentiation (Fst) and neutrality tests of Tajima D and Fu's Fs to assess the genetic differentiation among the populations and deviations from selective neutrality. A chi-squared test was used to show the significance of differences in the


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haplotype distributions. A BLAST search was performed against GenBank sequences to identify genetically related published haplotypes (Fig. 2). Genealogical relationships among the haplotypes were constructed using a median joining (MJ) network of *COI* haplotypes generated in NETWORK ver. 4.6 (40) with a 95% probability. To reveal the evolutionary relationships between individuals from the Russian populations and from known established populations in Europe, the New World and Asia, our data were combined with previously published *COI* data: 6 haplotype sequences found in this study plus 26 genetically related sequences from GenBank were used in this analysis (Fig. 2).

## Results

### The cytochrome c oxidase I gene diversity

A 1317bp fragment of the *COI* gene was sequenced from the 131 mosquitos, 10–34 specimens for each of 7 localities (Table 1). The likelihood of amplification of the pseudogene was rejected as no insertions, deletions or stop codons were found in any of the samples. Eight polymorphic nucleotide sites and six haplotypes (H) were detected, and two haplotypes were unique (Table 2). Data on the nucleotide diversity (Pi), the average number of nucleotide differences (K) and the observed haplotype diversity (Hd) are shown in Table 3. The highest values of Hd (0.757) and Pi (0.00182) were found in the Csa zone (Table 3). No obvious departure from neutrality was found in the investigated populations (Table 3), although Tajima's D statistic values were slightly positive (0.89987, P> 0.10) for the local population from the Csa zone and slightly negative for the local population from the Cfa-2 zone (-1.21122, P> 0.10) (Table 3). Tajima's D and Fu's Fs test values for all populations were not significant.

Among the six *Ae. albopictus COI* haplotypes discovered in our study, four were previously known in *Ae. albopictus* populations worldwide. The *COI* haplotypes designated in our work as H1, H2 and H5 correspond to H03, H17 and H54, respectively (25). Haplotype H3 is identical to the 26_J-Wa1 (41) and H79 (27). Two haplotypes, H4 and H6, are described for the first time. Thus, our results show that *Ae. albopictus* mosquitos collected in Russia in 2018 are related to populations involved in the worldwide expansion of this species through temperate regions.

### Population structure and differentiation

Four out of six *COI* haplotypes were shared by at least two local populations (Table 1). In the Cfa zone, haplotype H1 dominated in all the sampled localities, with the highest frequency recorded in the Cfa-3 subzone (85%, 29/34), followed by Cfa-2 (73%, 32/44) and Cfa-1 (68%, 19/28). In the Csa zone, only 20% of individuals had haplotype H1, which was significantly lower than in the Cfa zone ($\chi 2= 7.5902$, $p< 0.05$). No significant differences were observed in the distribution of haplotype H2, which was detected in 19% of individuals. The H3 haplotype was found in 10.7% (14/131) of individuals, of which 71.4% were collected in Novorossiysk and its surroundings, i.e. in the Csa zone. The difference in the distribution of the H3 haplotype in the Csa zone compared with that in the Cfa zone was significant ($\chi 2= 15.6415$, $p< 0.01$). The remaining haplotypes were rare (Table 1). Five haplotypes, H1-H5, were detected in *Ae. albopictus* sampled along the seacoast both in Cfa-1 and Csa zones, and only two haplotypes, H1 and H2, were found in individuals sampled in the steppe zone of Krasnodar krai (Cfa-3).

No genetic differentiation (Fst) was observed between the samples collected in the three Cfa climatic subzones. The migration (Nm) values between the Cfa-1, Cfa-2 and Cfa-3 local populations indicate frequent gene exchange between these populations (Table 4). The samples from the Csa zone differed significantly from those from the Cfa zone (Table 4).

### Phylogenetic analysis

The network of mitochondrial haplotypes was generated from six *COI* haplotypes obtained


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in our study and 26 identical, close related (differed by one nucleotide substitution) and connecting sequences from GenBank (Fig. 2). The H1 haplotype is the most widely distributed in Russia and identical sequence was found in Asia (Japan, Taiwan, and China), America (the USA and Canada) and Europe (Albania, Italy, Greece and Portugal). The sequence identical to H2 was also reported from China, Taiwan, the USA, Canada and Italy. The H3 was found in Japan and in the USA, whereas the H5 was revealed in the USA. The unique H4 and H6 haplotypes are connected by one mutation with H3 and H5, respectively. A total 32 *COI* haplotype sequences form two clusters in the network diagram. The first cluster includes the haplotypes H1 and H2 distributed in Europe and in other continents. The second cluster contains H3-H6 and *COI* haplotypes found mainly in the northeastern United States and Canada. The populations of Italy are the most studied in terms of the genetic diversity of *Ae. albopictus*, but no individuals with H3-H6 haplotypes were found here. Blast search based on the short and widely studied 450bp 5' *COI* gene fragment revealed that haplotypes H1 and H2 are distributed globally, including local European populations in Italy, Albania, Romania, Portugal, Serbia, Greece, Turkey and Abkhazia (GenBank acc. numb. JF 810659, HF912379, HQ906848, MG198595-600, JQ 412504-6, LN808745-46, MK518354, MK 995313). This short fragment of *COI* sequences, BARCODE fragment, is considered insufficient for phylogenetic analysis of *Ae. albopictus*, nevertheless haplotypes H3-H6 are quite different from H1 and H2 in this 5' gene fragment as well. These nucleotide and amino acid differences are shown in Table 2 including variable nucleotide positions in sites 72, 103, 110 and 276.

**Table 1.** Collection information and distribution of *Aedes albopictus* mtDNA *COI* gene haplotypes in Russia and Abkhazia

| | Localities | Climate zone/ subzone | Mean January Temp. (T°C) | Annual rainfall (mm) | No. of studied specimens | No. of specimens with haplotypes: | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | H1 | H2 | H3 | H4 | H5 | H6 |
| **1** | Abkhazia | Cfa-1 | ≥ 6,0 | 1200-1400 | 12 | 7 | 5 | | | | |
| **2** | Sochi | Cfa-1 | ≥ 6,0 | 1200-1400 | 16 | 12 | 1 | 3 | | | |
| **3** | Mountains-1 | Cfa-2 | ≥ 2,0 | 1200-1000 | 10 | 8 | 2 | | | | |
| **4** | Mountains-2 | Cfa-2 | ≥ 2,0 | 800-1000 | 34 | 24 | 6 | 1 | | 1 | 2 |
| **5** | Krasnodar | Cfa-3 | ≥ 0,0 | 600-800 | 21 | 19 | 2 | | | | |
| **6** | Adygeya | Cfa-3 | ≥ 0,0 | 600-800 | 13 | 10 | 3 | | | | |
| **7** | Novorossiysk | Csa | ≥ 2,0 | 400-600 | 25 | 5 | 6 | 10 | 1 | 3 | |
| **Total** | | | | | 131 | 85 | 25 | 14 | 1 | 4 | 2 |

**Table 2.** Variable sites of the *Aedes albopictus COI* gene sequences

| Haplotype | No. of individuals | Variable nucleotide sites* | | | | | | | | AA** | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 72 | 103 | 110 | 276 | 732 | 861 | 888 | 1002 | 35 | 37 |
| **H1** | 85 | T | A | T | T | A | C | T | T | I | I |
| **H2** | 25 | . | . | . | . | . | T | . | . | . | . |
| **H3** | 14 | . | G | . | . | G | T | . | C | V | . |
| **H4** | 1 | . | G | . | . | G | T | C | C | V | . |
| **H5** | 4 | . | . | C | C | . | T | . | . | . | T |
| **H6** | 2 | C | . | C | C | . | T | . | . | . | T |

*Dots denote identity with the reference sequence (as in Zhong et al. 2013, GenBank accession no. JQ004525), and the base letters denote substitutions; **Variable amino acid sites


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**Table 3.** Estimates of genetic diversity for the mtDNA *COI* region for populations of *Aedes albopictus*

| Climate zone / subzone | No. | S | H | Hd (±SD) | K | Pi (±SD) | Tajima's D | Fu's *Fs* |
|---|---|---|---|---|---|---|---|---|
| **Cfa-1** | 28 | 4 | 3 | 0. 5 (0.091) | 1.048 | 0.0008 (0.00024) | 0.05021 | 1.674 |
| **Cfa-2** | 44 | 7 | 5 | 0.445 (0.081) | 0.891 | 0.00068 (0.00019) | -1.21122 | -0.548 |
| **Cfa-3** | 34 | 1 | 2 | 0.258 (0.086) | 0.258 | 0.0002 (0.00019) | 0.08512 | 0.555 |
| **Csa** | 25 | 7 | 5 | 0,757 (0.051) | 2,393 | 0.00182 (0,00016) | 0.89987 | 1.609 |
| **Total** | 131 | 8 | 6 | 0.534 (0.043) | 1.294 | 0.00098 (0.00013) | -0.27687 | 0.706 |

No.- number of tested specimens, S- number of polymorphic sites, H- number of haplotypes, Hd- haplotype diversity, K- average number of nucleotide differences, Pi- nucleotide diversity (PiJC), Tajima's D and Fu's Fs statistics - neutrality tests, (Not significant, P> 0.10)

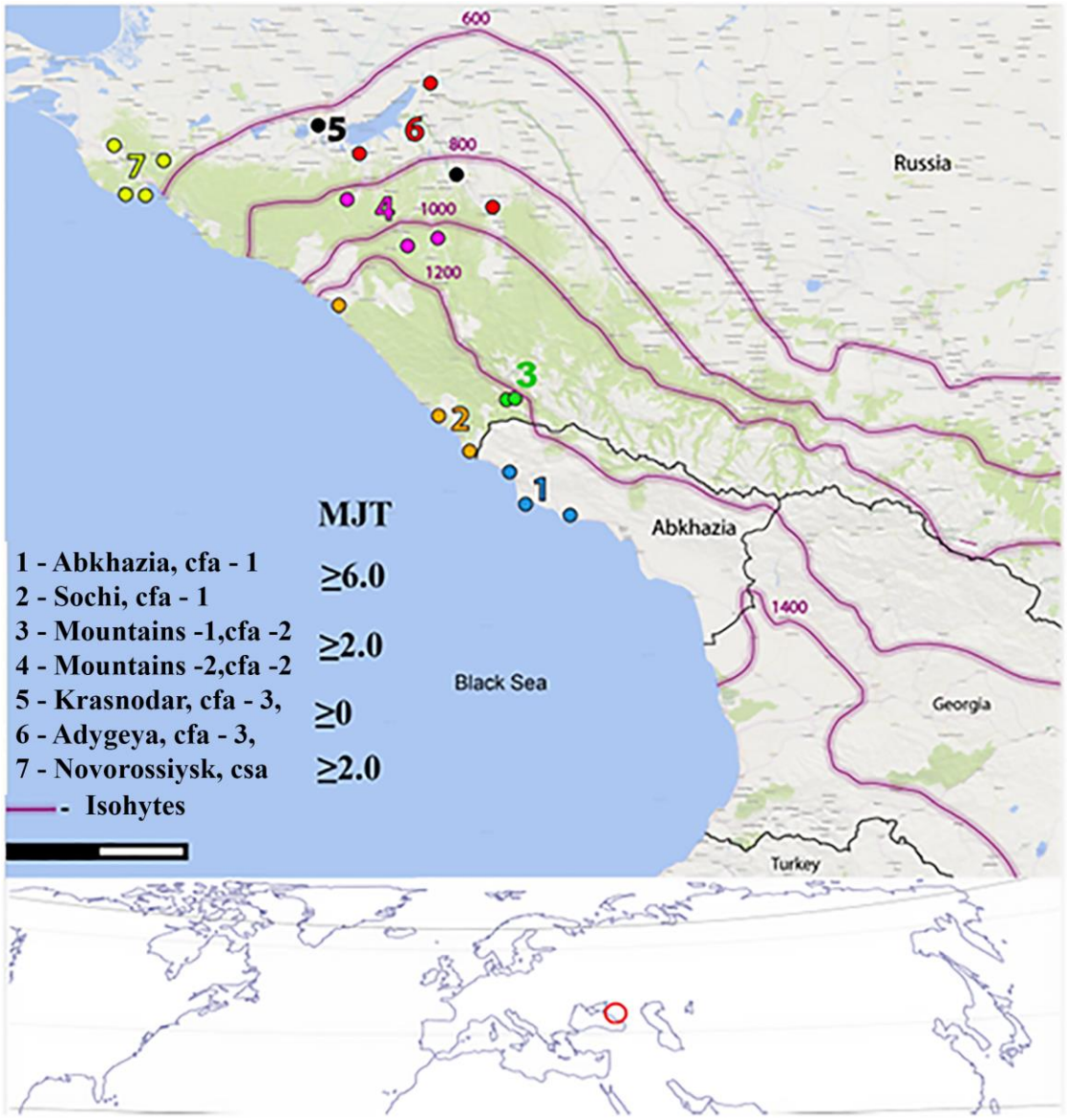


**Fig. 1.** Map of the study area and sample sites. Isohytes show annual rainfall (mm), MJT - Mean January Temperature (T°C). The location of the region on the world map (below) is shown with a red circle


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**Table 4.** Pairwise differentiation (FST, below the diagonal), and gene flow (Nm, above the diagonal) among populations of *Aedes albopictus* from different climatic zones/subzones

| Climate zone / subzone | Fst (white) and Nm (gray) estimates | | | |
|---|---|---|---|---|
| | Cfa-1 | Cfa-2 | Cfa-3 | Csa |
| **Cfa-1** | | 38.59 | 3.86 | 0.82 |
| **Cfa-2** | 0.00644 | | 9.72 | 0.55 |
| **Cfa-3** | 0.06088 | 0.02507 | | 0.34 |
| **Csa** | 0.23388 ** | 0.31392 *** | 0.42646 *** | |

The significance of differences: ns - not significant; *, 0.01 < P < 0.05; **, 0.001 < P < 0.01; ***, P< 0.001. Estimates of Fst: Csa-Cfa-1 Chi2= 15,817, P-value of Chi2: 0,0033 (P< 0.01) (df= 4); Csa-Cfa2 Chi2= 28,263, P-value of Chi2: 0,0000 (P< 0.001) (df= 5); Csa-Cfa3 Chi2= 66, P-value of Chi2: 0,0000 (P< 0.001) (df= 4)

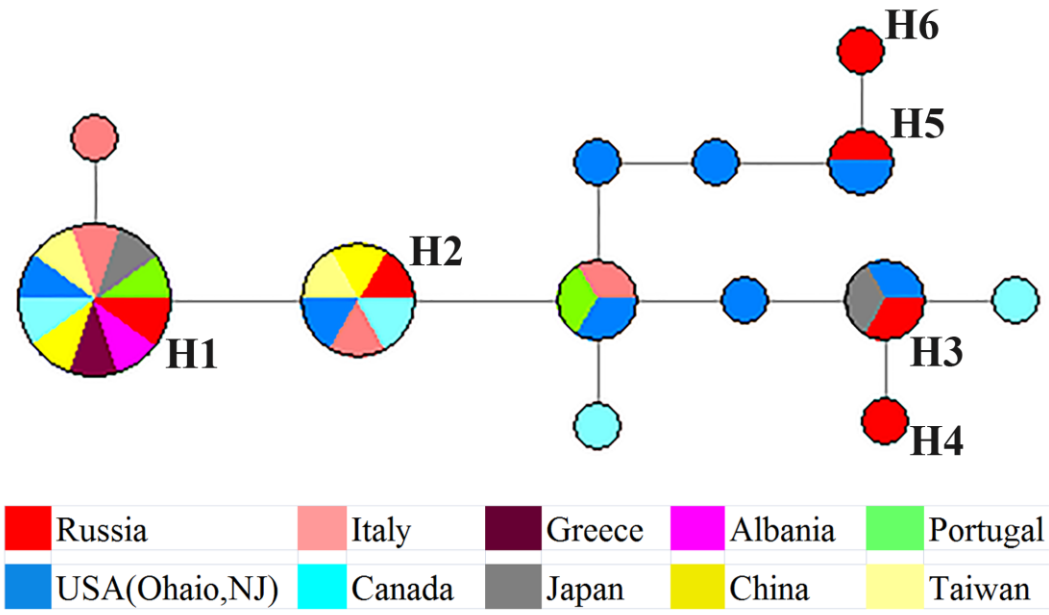


**Fig. 2.** The network diagram of the *Aedes albopictus COI* haplotypes obtained in this study and published data (25, 27, 28, 41). Colors correspond to different regions

## Discussion

A high degree of genetic variability within and between populations of *Ae. albopictus* is usually observed in endemic areas. Thirty-five *COI* haplotypes of *Ae. albopictus* were detected in the South Asian region, 33 in Malaysia, 42 in China, and 44 in Lao PDR (25, 26, 32, 34). In non-endemic areas where *Ae. albopictus* populations have recent origin and pass the bottleneck, the genetic diversity is low. In Europe, 11 *COI* haplotypes were found in Italy (25). Five *COI* haplotypes were found in Portugal, being confirmed by subsequent analyses based on *Ae. albopictus* mitogenomes (28). In studied populations, that spread along The Black Sea coast of the Caucasus, the genetic variability is also low that indicates its recent expansion.

No significant differentiation was found among populations in Cfa zone despite the climatic differences between Cfa-1, Cfa-2 and Cfa-3 subzones. The values of gene flow (Nm) and


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genetic differentiation (Fst) tests indicate the presence of a panmictic population in the Cfa zone (Table 4). Our conclusions are also supported by the results of the analysis of the genomes of individuals from Sochi and Krasnodar cities (Cfa zone), which showed that despite significant differences in the allele frequency spectrum, *Ae. albopictus* from Sochi and Krasnodar were closely related, had a common origin and formed a clade related to mosquitoes from Italy and Greece (42). The lack of genetic structure or isolation by distance were observed in many studies on *Ae. albopictus* populations worldwide independently of whether the populations are native or invasive. These data indicate ongoing and frequent gene flow among populations and seem to be due to a combination of low natural dispersion capabilities and a high level of human-mediated spread (19). Haplotypes H1 and H2 dominated in all three Cfa subzones (Table 1) and these haplotypes are also wide distributed both in the native regions of *Ae. Albopictus* (Taiwan and China) and in the areas of introduction (the USA, Canada, and Europe) (25, 27, 41). These results suggest two possibilities for the origin of the Russian *Ae. albopictus* populations in the Cfa zone. First, mosquitoes might have been introduced through seaports located on the Black Sea coast, such as Sochi, Russia and Batumi, Georgia from areas where *Ae. albopictus* with *COI* haplotypes H1 and H2 are widely distributed, for example, from Southeast Asia, China or the USA. Maritime transportation of tires and ornamental plants is known as the main dispersal mechanism of *Ae. albopictus* (43).

On the other hand, we cannot completely rule out the possibility of European origin of *Ae. albopictus* Cfa population. In countries of the Black Sea basin the mosquito was recorded first in 2005 in Greece, then in 2011 in Turkey, Georgia and Abkhazia suggesting its rapid and successive dispersion along the eastern Black Sea coast. The previous genetic studies confirmed the identity of short fragments of *Ae. albopictus COI* sequences, 450bp barcording fragment, from Greece, Turkey, Serbia, Romania, and Abkhazia (4, 10, 30, 31, 44). The 1317 bp fragments, identical to our H1 and H2 sequences, were also detected in *Ae. albopictus* from Albania, Greece and Italy (25, 41). Thus, the emergence of the Russian population can be associated with the terrestrial spread of mosquitoes due to human activity. This mechanism of *Ae. albopictus* dispersion has been observed in France and Spain and was suggested for Romania (4). If the mosquitoes of the Russian population are of European origin, their vector competence to dengue, Chickungunya and Zika viruses may be high enough for local transmission in the case of the occurrence of infected people as it took place in Italy, France and other Mediterranean countries (6).

Our results show the presence of spatial isolation and limited gene flow between populations from Cfa and Csa climatic zones. In Csa zone the haplotype diversity is twice as high as in the Cfa subzones with H3 being the most common haplotype, which is currently only found in the USA, Ohaio (27) and Japan, Wakayama (41). Haplotype H5 is also found in Novorossiysk and solely in USA, New York (25). These data indicate the presence of genetic structure in Russian population. The *COI*-based population structure has been found in Portugal, China, and Laos and is usually explained by climatic factors. Though the presence of a genetic structure in our population can also be associated with adaptation to the dry Mediterranean climate of Novorossiysk, this explanation seems unlikely. The history of introduction of *Ae. albopictus* to Russia has only about 10 years, and in Novorossiysk mosquitoes were recorded only in 2016. This time is not enough to accumulate adaptive mutations which is known to take a long time to occur. The introduced populations are more likely to establish if they already possess alleles advantageous in the invaded environment (45). In Europe, the spread of the mosquito began after the importation in the 1990s in Italy, which became the source for the rest of the European populations (46). In subse-


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quent period, the mosquito has spread widely in the areas with a dry Mediterranean climate, but haplotypes H3 and H5 distributed in Novorossiysk population were found neither in neighbouring countries (Turkey and Greece) no in Europe in general. Presumably, these haplotypes are rare and either have not been introduced in Europe or have not a selective advantage over other haplotypes in the specified climate. This suggests the independent introduction of *Ae. albopictus* to Novorossiysk, the largest seaport in southern Russia.

## Conclusion

The key finding of this work is the existence of two spatially isolated genetic lineages in *Ae. albopictus* population in southern region of Russia. Phylogenic analysis indicated that one of the lineages is similar to *Ae. albopictus* from USA and Japan whereas the second lineage is related to *Ae. albopictus* from Mediterranean countries. Our data for the first time confirm the hypothesis that the emergence of *Ae. albopictus* population in southern region of Russia may be associated with the terrestrial spread of mosquitoes from the well-established European population due to human activity. This finding is important for predicting the spread of invasive *Ae. albopictus* mosquitoes and the subsequent distribution of the medically important arboviruses that they transmit. Further research is needed to confirm this hypothesis. In the future, we plan to clarify the origin of the Russian populations using mitogenome analysis. The conclusions of mitogenome analysis do not contradict those of single *COI* gene analysis, but enhance the ability to identify population diversity and move us closer to understanding its origins.

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## Ethical considerations

The ethics committee of Vavilov institute of General Genetics has proved the study.

## Conflict of interest statement

The authors declare there is no conflict of interests.

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