

## Original Article

# Detection of the Invasive Mosquito Species *Aedes (Stegomyia) aegypti* and *Aedes (Hulecoeteomyia) koreicus* on the Southern Coast of the Crimean Peninsula

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## Abstract

**Background:** The incidence and area of arbovirus infections is increasing around the world. It is largely linked to the spread of the main arbovirus vectors, invasive mosquito of the genus *Aedes*. Previously, it has been reported that *Aedes aegypti* reemerged in Russia after a 50-year absence. Moreover, in 2011, *Ae. albopictus* was registered in the city of Sochi (South Russia, Black Sea coast) for the first time. In 2013, Asian *Ae. koreicus* was found in Sochi for the first time.

**Methods:** Mosquitoes were collected using the following methods: larvae with a dip net, imago on volunteers and using bait traps. The mosquitoes were identified using both morphology and sequencing of the second internal transcribed spacer of the nuclear ribosomal RNA gene cluster.

**Results:** In August 2016, *Ae. koreicus* larvae and imago and a single male of *Ae. aegypti* were found on the southern coast of the Crimean Peninsula, where they were not registered before. Newly obtained DNA sequences were registered in GenBank with the accession numbers MF072936 and MF072937.

**Conclusion:** Detection of invasive mosquito species (*Ae. aegypti* and *Ae. koreicus*) implies the possibility of their area expansion. Intensive surveillance is required at the Crimean Peninsula to evaluate the potential for the introduction of vector-borne diseases.

**Keywords:** Mosquito monitoring; Arbovirus vectors; *Aedes aegypti*; *Aedes koreicus*; DNA

## Introduction

At present, the invasive species *Aedes (Stegomyia) aegypti*, *Aedes (Stegomyia) albopictus* and *Aedes (Hulecoeteomyia) koreicus* are spreading across the world and have been registered in Caucasian coast of the Black Sea in southern Russia since 2011 (1-4). All of them are known as vectors of human pathogens (4). Invasive *Aedes* species have adapted its habitat preferences to human made containers, such as water storage tanks, discarded tires and jars, and water-filled pots (4). Due to the changing climate and the introduction of artificial hatching habitats, it was expected that these mosquito species would also spread to the southern coast

of Crimea, which has a climate very similar to Caucasus. In 2010, an entomological survey in the southern coast of Crimea found 15 blood-sucking mosquito species, namely three species of the genus *Anopheles* (*An. claviger*, *An. maculipennis*, *An. plumbeus*), 3 species of the genus *Culex* (*Cx. hortensis*, *Cx. pipiens*, *Cx. territans*), five species of the genus *Aedes* (*Ae. refiki*, *Ae. cantans*, *Ae. dorsalis*, *Ae. cataphylla*, *Ae. geniculatus*), three species of the genus *Culiseta* (*Cs. annulata*, *Cs. longiareolata*, *Cs. morsitans*) and *Uranotaenia unguiculata* (5). The coastal area of Crimea, especially the southern coast, is densely populated and attracts ap-

proximately five million tourists each year. The goal of the present study was to survey the southern coast of Crimea on the presence of invasive mosquito species.

## Materials and Methods

Mosquito collection was carried out in August, 2016. Mosquito sampling sites are shown in figure 1. The larvae were collected with a dip net, while the imago were collected either on volunteers using sucking tubes or “Electrofrog” bait traps (LMD-Komplekt Plus, Russia).

Mosquito sampling sites had different conditions. Priboy vacation resort (Number 1 in Table 1, Fig. 1) is located 300–500 meters to the northwest of Sakscoe Lake, one kilometer to the east is the resort city Saki, a town with well-developed residential areas. Frunzenets vacation resort (Number 2 in Table 1, Fig. 1) is located one kilometer away from a village on the shores of Lake Bagaily (Number 7 in Table 1, Fig. 1), which were overgrown with reeds and the inshore depth reached 1 meter. Mosquito larvae were collected in the thickets of the reeds. Departure of imago from the lake was registered. Imagoes were collected in the peak mosquito activity period from 8:30PM to 9:30PM.

The mosquitoes were identified by morphological key (7) and DNA sequencing. The most reliable characteristics for *Ae. koreicus* larvae were the form and number of comb scales on the lateral view of larval abdominal segment (Fig. 2A), position of the siphonal tuft and the number of the pectin teeth (Fig. 2B). Imago *Ae. koreicus* show a black and white pattern due to the presence of white scale patches on a black background and have clear longitudinal lines on the scutum (Fig. 2C). Two narrow white dorsocentral stripes are characteristic for *Ae. aegypti* (Fig. 2D).

DNA extraction was performed using Diatoms DNA prep kit (Isogen, Moscow). The Encyclo PCR kit (Evrogen, Moscow) and primers complementary to 5.8S and 28S rRNA

genes (8) were used for amplification of the second internal transcribed spacer (ITS2) region of rRNA genes. Amplification products were visualized in 1% agarose gel and purified from the gel using a clean-up kit (Evrogen, Moscow). Sequencing was performed using ABI PRISM® 310 Genetic Analyzer with BigDye® Terminator kit (Applied Biosystems, USA). The obtained DNA sequences were registered in GenBank (accession numbers MF072936 and MF072937). The internet resource <https://www.gismeteo.ru/diary/> was used for climate analysis.

## Results

Imago and larvae of eight mosquito species, 661 individuals in total, were collected on the Crimean Black Sea coast and in Simferopol (Fig. 1, Table 1).

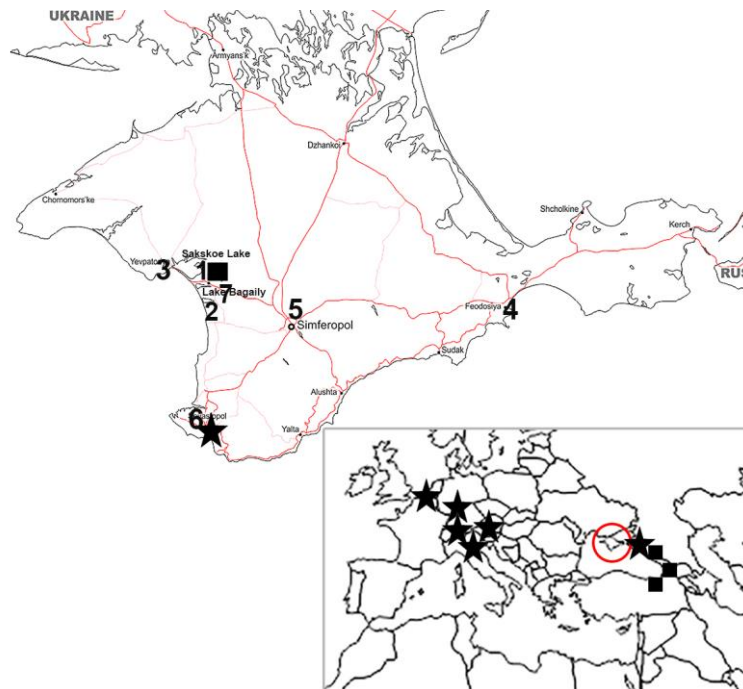
In Simferopol, *Cx. pipiens* was collected in water storage tanks. In Lesnoy Village (№6 in Table 1, Fig. 1), located close to the town of Sevastopol, mosquitoes were collected in an artificial concrete basin with an area of 23m<sup>2</sup> and a depth of 0.5m. In this artificial basin, the larval density per 1m<sup>2</sup> was the highest: *Ae. koreicus* – 238 individuals; *Cs. longiareolata* – 256; and *Cx. pipiens* – 17.

Among them, one male *Ae. aegypti* was discovered at Priboy vacation resort. Morphological identification was confirmed by DNA sequencing. This *Ae. aegypti* ITS2 DNA sequence was deposited to GenBank (accession number MF072936). The ITS2 nucleotide sequence of the Crimean *Ae. aegypti* was identical to the one from Abkhazia, 2012 (MH142316) and Adler (Russia), 2013 (MH142319). There was one nucleotide substitution in ITS2 (99% identity) between *Ae. aegypti* found in Crimea (MF072936) and *Ae. aegypti* sequences registered in GenBank: Rockefeller strain KF471581-KF471583; JX423805 and JX423807 from Saudi Arabia; KU497616 from China; and KF471579 from New Caledonia.

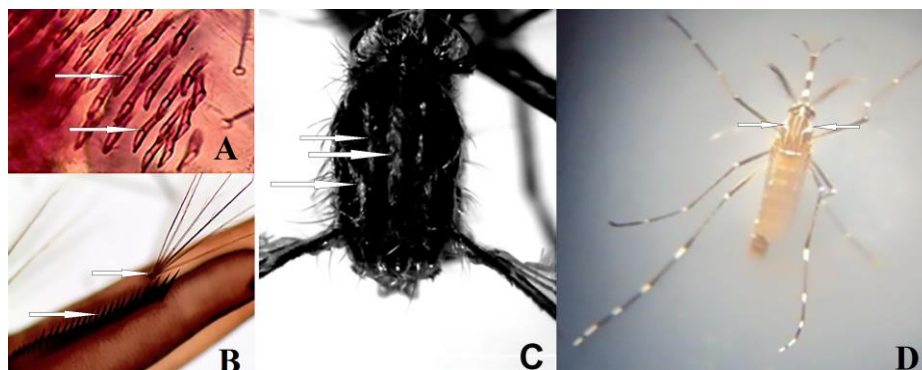
Larvae of the species *Ae. koreicus* were found

close to Sevastopol in Lesnoy Village (Table 1), in an artificial concrete basin. *Aedes koreicus* imago were found next to this pond. The sequence of the ITS2 genome region of *Ae. koreicus* larvae found in Crimea was identical to the DNA sequence of *Ae. koreicus* from Belgium (KF471636 and JF430391) and Sochi (HG763830). The Crimean *Ae. koreicus* DNA sequence was deposited in GenBank with the accession number MF072937.

In addition to these two invasive species, representatives of six autochthonous mosquito species were registered in the studied area. Imagoes sampled at all locations were mainly represented by the species *Cx. pipiens*, *Cx. modestus*, and a few individual *Coquillettidia richiardii* and *Ae. caspius* (Table 1). *Culiseta longiareolata* and *Cx. pipiens* larvae were found in Lesnoy Village (Table 1).



**Fig. 1.** Map of Crimean Peninsula with the mosquito sampling locations indicated as in Table 1. The map of Europe with the current known established populations of *Aedes koreicus* and *Ae. aegypti* in Europe (ECDC; 2020. Available from: <https://ecdc.europa.eu/en/disease-vectors/surveillance-and-disease-data/mosquito-maps>), Crimean Peninsula indicated with a circle. Locations of *Ae. aegypti* collection are shown with a square, *Ae. koreicus* - with a star.



**Fig. 2.** Diagnostic characters of *Aedes koreicus* larva (A, B) and female (C), *Ae. aegypti* male (D)

**Table 1.** Composition of adults and larvae of 661 collected mosquitoes

Number on the map	Sample site	Coordinates	Mosquito species							
			<i>Ae. aegypti</i>	<i>Ae. koreicus</i>	<i>Ae. caspius</i>	<i>Cx. pipiens</i>	<i>Cx. modestus</i>	<i>Cx. hortensis</i>	<i>Cq. richiardi</i>	<i>Cs. longiareolata</i>
1	Priboy	45°13'05"N 33°52'22"E	1♂		3♀	154♀ 5♂	157♀4♂		11♀	
2	Frunzenets	45°02'37"N 33°63'28"E			1♀	52♀3♂	90♀3♂		2♀	
3	Evpatoria	44°12'00"N 33°21'30"E				26♀	21♀			
4	Koktebel	44°57'35"N 35°14'52"E				3♀	3♀			
5	Simferopol	44°56'53"N 34°06'15"E				12♀10L				
6	Sevastopol (Lesnoy Village)	44°43'55"N 33°34'55"E		3♀29L		2L				33L
7	Lake Bagaily	45°02'37"N 33°63'28"E				8L	16L	9L		
Total number of individuals			1	32	4	275	294	9	13	33

## Discussion

Screening of mosquitos on the south coast of Crimean Peninsula found, among others, *Ae. aegypti* and *Ae. koreicus*, which have not been registered in Crimea before 2016 (5). In 2018, Kovalenko and Tikhonov (9) examined eight regions of the Crimea and collected seven *Ae. koreicus* females in Sevastopol and three in Simferopol. Although no *Ae. koreicus* larvae or breeding sites were found in this study, this data implied the spread of mosquitoes to the center of the peninsula. So, additional finding of *Ae. koreicus* imagoes in Crimea in 2018 confirms the establishment of *Ae. koreicus* population in Crimea.

Native areas of *Ae. koreicus* are Korean peninsula, China, Japan, and the Russian Far East. Recently, *Ae. koreicus* has been expanding to new areas due to exobiological plasticity. The species *Ae. koreicus* was first found in the European part of Russia in 2013 in the center of the town of Sochi (4, 9, 10). At present, *Ae. koreicus* has been registered in many European countries including Belgium, northern Italy, Hungary, Slovenia and Germany (11–18). It is known that *Ae. koreicus* can withstand lower winter temperatures and is drawn

to populated areas located near woods. Its larvae, as well as those of *Ae. aegypti* and *Ae. albopictus*, develop in artificial basins. Females usually attack in the open air, occasionally indoors. Mosquitoes overwinter in the egg stage. *Aedes koreicus* has shown vector competence in the field and in laboratory experiments for a number of pathogens including dengue, West Nile fever, Japanese encephalitis viruses and *Dirofilaria* (12, 19). Therefore, discovery of *Ae. koreicus* in Crimea marks a significant threat of the introduction and spread of vector-borne diseases.

Detection of a single *Ae. aegypti* male can be attributed to either breeding in the close-by residential area, which provides artificial reservoirs serving as breeding habitats, or an isolated introduction, possibly from the Black Sea coast of the Caucasian region, where these mosquitoes were recently registered (2, 4, 6). The latter possibility has been exemplified previously by discovery of a single *Ae. aegypti* male in Northwest England (53°30'42"N, 2°59'01"W) in 2014, where climate, temperature and humidity were generally unsuitable for this new invasive mosquito (20). In Cri-

mea, where the *Ae. aegypti* male was discovered in the present study, the highest temperature during the summer season was registered in August 2016. At the time of mosquito collection, the temperature reached 36 °C during daylight hours and 28–31 °C at night. The relative humidity in July and August (67–68%) was uncomfortable for these mosquitoes. During May–August, it rained for only 2 days and the total amount of precipitation over the year was approximately 400mm whereas in Sochi, where *Ae. aegypti* mosquitoes were previously registered, the annual rainfall exceeds 1500 mm. It is believed that *Ae. aegypti* distribution is limited to areas with a January isotherm of 10 °C and mean annual temperatures of 15 °C, whereas in Crimea in the winter 2015–2016 the lowest winter temperature reached -15 °C, and in subsequent years it was between -6 °C and -7 °C. Unfavorable conditions may indicate that *Ae. aegypti* was an occasional introduction to the peninsula. To our knowledge, there is no published data on findings of *Ae. aegypti* in Crimea (<https://ecdc.europa.eu>, 2018). At the same time, *Ae. aegypti* is well established on the Black Sea coast in Caucasus (4), and is now spreading along the Black Sea coast of Turkey and Georgia (6, 21).

In 2014, we suggested that *Ae. albopictus* mosquitoes were likely to appear in Crimea (22), but neither larvae nor imago of this species were discovered during the present study. Nevertheless, climate change and the detection of the more cold - sensitive *Ae. aegypti* highlights the risk of *Ae. albopictus* rooting in Crimea and calls for detailed entomological monitoring on the peninsula. The predominance of *Cx. pipiens* and *Cx. modestus* imagoes can be explained by the proximity of the sampling areas Frunzenets and Priboy to lakes (Fig. 1), which are a favorable environment for the development of the genus *Culex* larvae, as evidenced by the presence of *Culex hortensis* larvae at Bagaily Lake (Table 1). The absence of *Cx. hortensis* imago among the collected samples (as compared with the

larvae samples) is due to the fact that these species are reluctant to attack humans and spend most of the time in caves and similar sheltered environments.

## Conclusion

In the current study, larvae breeding habitats for *Ae. koreicus* and *Ae. aegypti* imago have been found on the southern coast of the Crimean Peninsula for the first time. The *Ae. aegypti* species was represented by a single male specimen; therefore, it is not clear if it has yet been established in Crimea. Our study indicates that *Ae. koreicus* is locally established in Crimea and its northward expansion is expected, highlighting the challenges for vector surveillance and control programs.

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