

For the Barcode of Life Data System see <http://www.boldsystems.org>

For the Basic Local Alignment Search Tool see <https://blast.ncbi.nlm.nih.gov>

See Online for appendix

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## ***Aedes aegypti* carrying triple knockdown resistance mutations in Beijing, China**

Pandemic arthropod-borne viruses have emerged as a global public health concern over the past four decades.<sup>1</sup> *Aedes aegypti* and *Aedes albopictus* are vectors of several important arboviral diseases, including yellow fever, dengue, and Zika virus. The detection of *A albopictus* in southern England, UK,<sup>2</sup> and the re-emergence of *A aegypti* in Egypt<sup>3</sup> highlighted severe situations of vector introduction. Here, we report for the first time the unexpected presence of *A aegypti* in Beijing, China, a city with a typical north-temperate climate.

In our efforts to investigate insecticide resistance-associated mutations in *A albopictus*,<sup>4</sup> we discovered three *A aegypti* in a sample of 56 *Aedes* spp mosquitoes that were trapped in carbon dioxide in the Grand Canal Forest Park, Beijing, in July, 2017; *A aegypti* was not observed in samples from 16 other locations in Beijing. The identification of the three mosquitoes as *A aegypti* was supported by sequence data for four genes: acetylcholinesterase (*ace*), voltage-gated sodium channel (*vgsc*), mitochondrial cytochrome oxidase subunit I (*coi*), and ribosomal DNA

internal transcribed spacer. A search with the *coi* sequence (609 bp) against the Barcode of Life Data System identified the mosquitoes as *A aegypti*. Searches on the US National Institutes of Health's Basic Local Alignment Search Tool revealed entries of *A aegypti* that were identical to our sequences, confirming that the three samples were *A aegypti* (appendix).

Notably, all three *A aegypti* mosquitoes carried insecticide resistance-conferring mutations in *vgsc*—namely, Ser989Pro (homozygous), Val1016Gly (homozygous), and Phe1534Cys (heterozygous)—suggesting they had a high level of resistance to pyrethroids. The knockdown resistance mutations of *vgsc* might facilitate the dispersal of *A aegypti* because resistant mosquitoes can survive from possible pyrethroid exposure during their journey. The extremely high similarity in gene sequences between the three mosquitoes and those from southeast Asia (appendix) indicates that these *A aegypti* mosquitoes might originate from southeast Asia.

Because three *A aegypti* mosquitoes with highly similar gene sequences were detected only in *Aedes* spp samples collected in the Grand Canal Forest Park, and the presence of *A aegypti* was not documented in 91 *Aedes* spp mosquitoes collected in August and September, 2018, and 146 mosquitoes collected in August, 2019, from the same location, we presume that *A aegypti* has not colonised Beijing. Beijing is a huge city with a population of more than 20 million people, and with busy national and international transport networks, the epidemiological risk of aedes-vector-borne diseases is high.

The discovery of *A aegypti* in the Grand Canal Forest Park in 2017 indicates the possibility of the occurrence of this pest in Beijing, at least during warm months. We highly encourage a more vigilant and extensive surveillance programme to monitor the possible occurrence and dissemination of *A aegypti* in Beijing. To prevent introduction of this potent

disease vector, there should be strict inspections at the various ports of entry.

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## ***Vibrio vulnificus* casualties during the American Civil War**

An estimated 700 000 soldiers belonging to the Union Army and the Confederate States Army died in the 1861–65 American Civil War.<sup>1</sup> Many camp epidemics attributed to dysentery, typhoid fever, measles, smallpox, cholera, and malaria spread quickly among the troops, affecting the Confederate Army more and surely to some extent shaping the outcome of the war.<sup>2</sup>

We investigated samples taken from the unmarked graves of nine confirmed Confederate soldiers who died in the first 2 years of the war from camp diseases. The soldiers were in one Confederate state and were to be exhumed and moved to an undisclosed location. Using the dental