The First Record of the Invasive Mosquito Species Aedes Albopictus in Chisinau, Republic of Moldova, 2020

Tatiana Sulesco (tatiana_sulesco@yahoo.com)
Institute of Zoology https://orcid.org/0000-0002-8287-1461

Galina Busmachiu
Institute of Zoology

Unchana Lange
BNITM: Bernhard-Nocht-Institut fur Tropenmedizin

Jonas Schmidt-Chanasit
BNITM: Bernhard-Nocht-Institut fur Tropenmedizin

Renke Lühken
BNITM: Bernhard-Nocht-Institut fur Tropenmedizin

Short report

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Abstract

Background

In Europe, *Ae. albopictus* is an important vector of chikungunya virus, *Dirofilaria* nematodes and was involved in local autochthonous circulation of dengue virus and Zika virus. Due to the ongoing spread, a targeted field surveillance at potential points-of-entries for *Aedes* invasive mosquitoes was initiated for the Republic of Moldova in 2020 as part of the transboundary “Aedes Invasive Mosquito COST Action project”.

Methods

In 2020, ovitraps were positioned at each of three locations: the border crossing to Romania in Leuseni (Hancesti region), Chisinau International Airport and Chisinau Botanical Garden.

Results

A total of 188 *Aedes* spp. eggs were collected at the Chisinau International Airport between August and September 2020. Twenty-three adults reared in the laboratory were identified morphologically as *Aedes albopictus* (Skuse, 1895) and twelve selected specimens were confirmed by molecular barcoding of the cytochrome oxidase subunit I gene region. In addition, one adult female of *Ae. albopictus* was caught at the same site with a manual aspirator from a human.

Conclusions

This is the first documented report of *Ae. albopictus* in the Republic of Moldova. The presence of immature and adult stages indicates the local reproduction of the species in the country. Therefore, it is crucial to extend and strengthen surveillance of the invasive *Aedes* mosquitoes to prevent *Ae. albopictus* and other exotic mosquito species from establishing in the Republic of Moldova.

Background

Aedes albopictus (Skuse, 1895), commonly known as Asian tiger mosquito, is an invasive mosquito species native to tropical and subtropical regions of Southeast Asia and the Indian Ocean (Paupy et al., 2009). Over the last four decades, the species rapidly expanded its distributional range worldwide including The Americas, Africa, Australia and Europe (Kraemer et al., 2015). Experimental and field data demonstrated that *Ae. albopictus* is a potential vector of more than 30 different pathogens (Paupy et al, 2009, Vanlandingham, 2016, Pereira-dos-Santos et al, 2020). In Europe, *Ae. albopictus* is an important vector of chikungunya virus with several outbreaks in Italy and France (Rezza et al., 2007, Venturi et al., 2016) and *Dirofilaria* nematodes (Cancrini et al., 2007). In addition, the species was involved in local autochthonous circulation of dengue virus (Schmidt-Chanasit et al., 2010, Succo et al., 2016) and Zika virus (Giron et al., 2019) in Europe.

Globalization with increasing international trade and travel facilitate the spread of *Ae. albopictus*. Due to its ecological plasticity (Paupy et al., 2009), *Ae. albopictus* invaded and became established in thirty countries in Europe including the neighboring regions of the Republic of Moldavia in the Mediterranean Basin: the Thrace region of Turkey and the eastern Black Sea coast (Bellini et al., 2020, Akiner et al., 2016, Ganushkina et al, 2016). Recently, the species was introduced in Crimean peninsula, the northern Black Sea coast (Kovalenko et al., 2020). The first report of *Ae. albopictus* in Romania, the neighboring country to the Republic of Moldova, was in Bucharest with the country's main international airport, 2012 (Prioteasa et al, 2015). Further sampling efforts demonstrated the spread of *Ae. albopictus* in the country with the closest positive site relative to the border of Moldova located in Constanta region (Fălcuță et al, 2020). Thus, a targeted field surveillance for potential points-of-entries for *Ae. albopictus* was initiated for the Republic of Moldova in 2020 as part of the "Aedes Invasive Mosquito (AIM) COST Action project" (https://www.aedescost.eu/).

Mosquito findings and identification

Field surveys were conducted at three locations, presenting potential routes of entry for invasive *Aedes* mosquito species to the Republic of Moldova. These include the border crossing to Romania in Leuseni, Hancesti region, (June 10th - October 16th 2020), Chisinau International Airport (July 05th - October 31st 2020) and Chisinau Botanical Garden (July 10th - October 16th 2020), known for introducing and growing tropical plants (Fig. 1). Conical shaped black plastic containers (height: 13 cm high, lower diameter: 9 cm, upper diameter: 13 cm) with a volume of about 1 L were used as ovitraps (Bellini et al., 2020). Each trap was filled two thirds with clean water and scratched tongue depressors (1.7 x 15 cm) were added as a egg-laying substrate for invasive *Aedes* species. Five ovitraps were positioned in each location in the shaded sites at a distance not less than 20 meters from each over (Fig. 1). The tongue depressors and water were collected from the traps and replaced with clean water and new tongue depressor every two weeks. All samples were transported to the laboratory of Entomology, Institute of Zoology in Chisinau for rearing of eggs and larvae to adults in trays containing dechlorinated water. Hatched larvae were fed with aquarium fish food (ASTRA Aquaristik GmbH, Osnabrück, Germany). Morphological species identification of larvae and adults was conducted with the keys in Becker et al. (2010).

Two mosquito taxa were collected from the ovitraps during the entomological surveys: *Culex pipiens* s.l./*Cx. torrentium* (205 individuals) and *Aedes* spp. (188 eggs) (Table 1). *Culex pipiens* s.l./*Cx. torrentium* was present at all three study locations, while *Aedes* spp. eggs were only collected at the Chisinau International Airport. Two ovitraps positioned in the forest square close to the airport collected a total of 188 *Aedes* spp. eggs. The first positive ovitrap (E_MD_AC5: Lat 46.938, Long 28.928) yielded 167 eggs: 72 eggs (21.8.), 38 eggs (5.9.) and 57 eggs (27.9.). The second trap (E_MD_AC2: Lat 46.936, Long 28.940) collected 21 *Aedes* spp. eggs on September 27. Twenty-three specimens (19 females and 4 males) were sucessfully reared from the *Aedes* spp. eggs to adult stage and identified as *Aedes albopictus* by larval and adult morphology (Fig. 2). Identity of *Ae. albopictus* was confirmed by molecular barcoding of the cytochrome oxidase subunit I gene region of twelve randomly selected specimens (Fang et al., 2017). All sequences have been entered into GenBank (accession no. MZ069031-MZ069042). In

addition, one female of *Ae. albopictus* was caught from human by manual aspirator during ovitraps inspection in the airport on September 27 (Fig. 1b). Three additional ovitraps (E_MD_AC6, E_MD_AC7, E_MD_AC8) were placed at the Chisinau International Airport at the end of September and surveillance continued through to October 31, but no further *Aedes* spp. eggs were collected.

Discussion And Conclusions

The introduction of *Ae. albopictus* in new territories causes major concerns in public and veterinary health, due to its severe nuisance and vectorial capacity for several pathogens like dengue virus, chikungunya virus and Zika virus (Rezza et al., 2007, Schmidt-Chanasit et al., 2010, Giron et al., 2019). The introduction of *Ae. albopictus* in Europe was facilitated by passive dispersion through the global transportation of tires (Adhami and Reiter, 1998, Dalla Pozza and Majori, 1992) and the import of *Dracaena* plants known as “lucky bamboo”, e.g. Netherlands (Scholte et al., 2007) or Bulgaria (Mikov et al., 2013). Further dispersal in Europe inside vehicles via highway systems was documented for Switzerland (Flacio et al., 2004), Germany (Pluskota et al., 2008), Spain (Collantes et al., 2015) or the United Kingdom (Medlock et al., 2017). However, only few studies have been dedicated to the role of European airports for the import of the exotic mosquito species with *Ae. albopictus* records for Schiphol airport, The Netherlands (Becker et al., 2013, Scholte et al, 2014, Ibáñez-Justicia et al, 2020, Horvath et al., 2021).

Surveillance of the presence/absence of invasive *Aedes* species at the potential points-of-entry in the Republic of Moldova demonstrated the presence of *Ae. albopictus* at the Chisinau International Airport. This finding underlindes the importance of air transportation for the dispersal of *Ae. albopictus* in Europe. This is the first documented report of *Ae. albopictus* in the Republic of Moldova and the presence of adult and immature stages indicates the local reproduction of the species. Further investigations of to clarify if this is a stable, established population. Nevertheless, with the increasing spread and population densities of *Ae. albopictus* in Europe, additional introductions have to be expected via air traffic or other routes of entry, which may allow long-term establishment. Therefore, it is crucial to extend and strengthen surveillance of invasive *Aedes* mosquitoes in order to prevent establishment and future arbovirus transmission in the Republic of Moldova.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analysed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

TȘ designed the study. TȘ, GB, UL and RL conducted the field and laboratory work. TȘ and RL prepared the manuscript. GB, UL and JSC contributed to the discussion and improvement of the manuscript. All authors read and approved the final manuscript.

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Tables

Table 1 *Aedes* spp. eggs with confirmed *Aedes albopictus* specimens and *Culex pipiens* s.l./ *Cx. torrentium* larvae collected from 15 ovitraps at three sampling locations in the Republic of Moldova,

2020.

Location	*Aedes* spp. eggs	Hatched *Aedes* spp. (confirmed as *Aedes albopictus* by morphology/tested and confirmed by COI barcoding)	*Culex pipiens* s.l./ *Cx. torrentium*
Airport, Chisinau			
E_MD_AC1			59
E_MD_AC2	21	0	
E_MD_AC5	167	23 (23/12)	
Botanical garden, Chisinau			
E_MD_CH3			28
E_MD_CH4			20
Leuseni, Hancesti			
E_MD_LE3			15
E_MD_LE5			83
Total	**188**	**23**	**205**

Figures

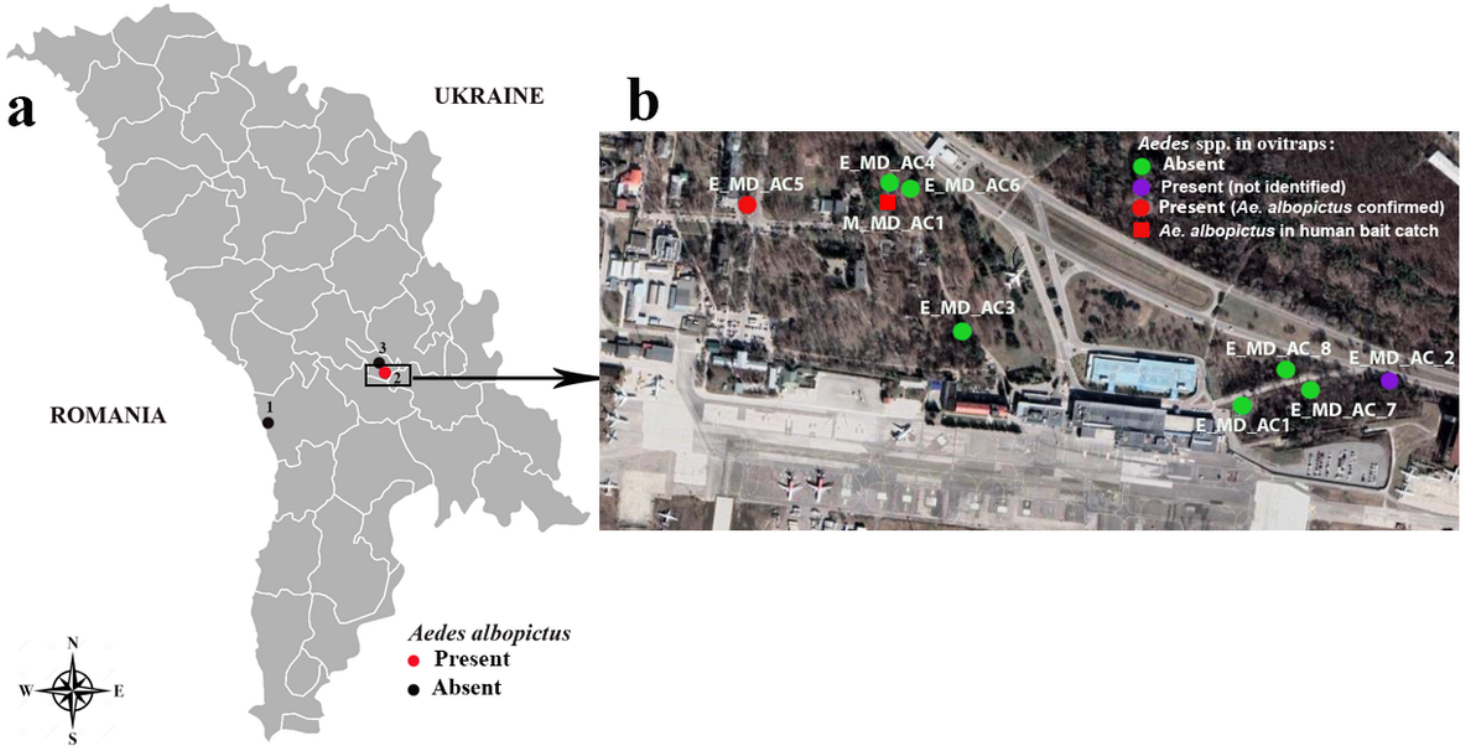

Figure 1

Study area. a - sampling locations in the Republic of Moldova: 1 - the border crossing to Romania in Leuseni; 2 – Chisinau International Airport; 3 – Chisinau Botanical Garden. b – sampling sites in the Chisinau International Airport. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

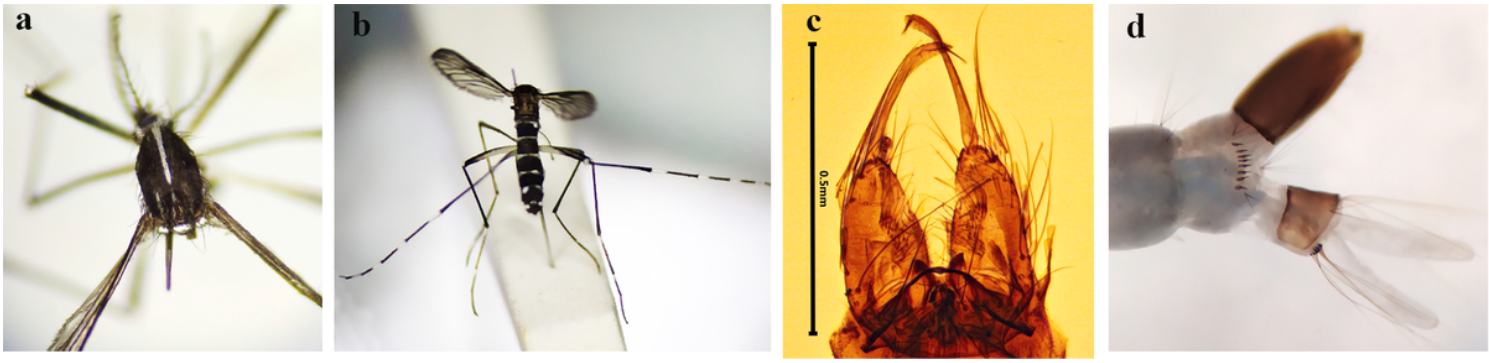

Figure 2

Specimens of Aedes albopictus collected at the Chisinau International Airport in 2020. a – adult female, pattern on scutum; b – adult female, dorsal view, c – male genitalia; d – fourth-instar larva.

Supplementary Files

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- graphicalabstract.png