Arrival of the Asian tiger mosquito, *Aedes albopictus* (Skuse, 1895) in Vienna, Austria and initial monitoring activities

Karin Bakran-Lebl1 | Carina Zittra2 | Josef Harl3 | Bita Shahi-Barogh1 | Andreas Grätzl1 | David Ebmer4 | Francis Schaffner5 | Hans-Peter Fuehrer1

1 Department of Pathobiology, Institute of Parasitology, University of Veterinary Medicine Vienna, Vienna, Austria
2 Department of Functional and Evolutionary Ecology, University of Vienna, Vienna, Austria
3 Department of Pathobiology, Institute of Pathology, University of Veterinary Medicine Vienna, Vienna, Austria
4 Veterinary Clinic Tiergarten Schönbrunn, Vienna, Austria
5 Francis Schaffner Consultancy, Riehen, Switzerland

**Abstract**

*Aedes albopictus* was recorded in Vienna, Austria, in August 2020 for the first time. The species was found to occur in three sites within the city; morphology-based monitoring was followed by DNA-barcoding. Mitochondrial COI barcode sequences recovered three different haplotypes, however this data does not reveal whether single or multiple introduction events have occurred. The vicinity of Viennese *Ae. albopictus* sites to major traffic routes highlights the importance of passive transport for range expansion of this species.

**Keywords**

*Aedes albopictus*, Austria, citizen science, invasive mosquitoes

**1 | INTRODUCTION**

In recent decades, several alien mosquito species have been introduced to Europe, primarily through the global transport of goods. In the case of suitable climatic conditions these species were able to form stable populations and further expanded their distribution (Medlock et al., 2012, 2015). Alien mosquito species pose a potential threat, as these newly introduced species may also carry exotic pathogens (Medlock et al., 2012).

Of particular importance is the Asian tiger mosquito (*Aedes albopictus*), which is not only an annoying day-active biter, but is also able to transmit a broad range of pathogens such as chikungunya, dengue and Zika, which cannot be transmitted by native mosquito species in Austria (ECDC, 2021a; Medlock et al., 2012). *Aedes albopictus* was introduced to Europe mainly with freight transports (especially used tyres and lucky bamboo; Scholte & Schaffner, 2007). From southern Europe, where it has become rapidly established, adults have been passively transported further north in cars and trucks (Eritja et al., 2017; Scholte & Schaffner, 2007). In Germany and Switzerland, this mosquito species has been found in particular along freeway routes from southern Europe (Becker et al., 2013; Flacio et al., 2016; Müller et al., 2020).

Since 2012, single records of *Ae. albopictus* in southern and western parts of Austria, mostly within a long-term mosquito surveillance program (Fuehrer et al., 2020; Schoener et al., 2019; Seidel et al., 2012), have been limited to small towns and major highway stations, indicative of continuous introduction and local reproduction. At present, there...
is no evidence for overwintering or established populations in Austria. Here, we report the first finding of this species in Vienna (Austria), one of the largest cities within the European Union.

2 | MATERIAL AND METHODS

On 20 August, 2020, an unusual mosquito specimen was collected by a citizen in an allotment garden in the vicinity of the Danube River in the Leopoldstadt, the second municipal district of Vienna. This citizen had participated in 2018 in a citizen science project whose purpose had been to detect alien mosquito species, using self-made traps in Viennese allotment settlements. The mosquito was sent to the Institute of Parasitology at the University of Veterinary Medicine Vienna, the institution that had conducted this previous citizen science project. There, this specimen was identified as *Aedes albopictus* (Skuse, 1895) by morphological characteristics (Becker et al. 2010), this representing the first record of this species in Eastern Austria.

Another *Ae. albopictus* specimen was collected on 3 September with a carbon-dioxide-baited BG-Sentinel trap (BGS; Biogents®, Regensburg, Germany) in Donaustadt, the 22nd municipal district of Vienna, about 3.3 km from the first site, during a routine mosquito monitoring program (conducted by the University of Veterinary Medicine Vienna on behalf of the City of Vienna). On 11 September, a specimen collected about 3.9 km from the site of the first record in Donaustadt was reported photographically through the app ‘Mosquito alert’ (www.mosquitoalert.com/en), implemented through the AIM-COST Action pan-European surveillance activities (www.aedescost.eu). This specimen was judged by three independent experts to be ‘probably *Ae. albopictus*’ (Figure 1 shows the collection sites).

Following the first report of *Ae. albopictus* in Leopoldstadt, a brief investigation of the allotment garden was carried out to assess whether
this was a stray individual, or if *Ae. albopictus* was already breeding there. We targeted three locations around the original collection site: location A, the garden of the homeowner who collected the specimen, was monitored with 1 BGS trap baited with carbon dioxide and a specific lure (BG-Sweetscent, Biogents®, Regensburg, Germany) and 2 ovitraps (black 1 L cups filled with ~750 ml water, with a wooden tongue depressor as oviposition support); location B, ~600 m from location A and ~110 m from location C was monitored with an equivalently equipped BGS trap and 2 ovitraps; finally, location C, ~520 m from location A, was monitored with 2 ovitraps. The traps were run for one week starting on 8 September; the trap nets of the BGS traps were replaced once on 11 September. While the traps were being set up, one *Ae. albopictus* male was collected with an aspirator.

We performed DNA-barcoding targeting the mitochondrial COI barcode fragment as reported previously (Hebert et al., 2004) to confirm morphological identifications of adults and mosquito eggs. DNA was extracted with the ’DNeasy® Blood and Tissue’ DNA isolation kit (Qiagen, Hilden, Germany) following homogenisation with three 1.4 mm ceramic beads (Precellys Ceramic Kit 2.8 mm/1.4 mm, Peqlab, Erlangen, Germany) on a TyssueLyser II (Qiagen, Hilden, Germany). Conventional PCR using primers LepF1 and LepR1 was performed as reported previously (Hebert et al., 2004) and PCR products were sent to LGC Genomics (Berlin, Germany) for bidirectional sequencing. The sequences were manually aligned and carefully checked by eye using BioEdit 7.0.5.3 (Hall, 1999). One sequence of each haplotype was uploaded to NCBI GenBank (accession numbers MW457632–MW457634).

To investigate the origin of the specimens found DNA haplotype networks were created. NCBI GenBank was mined for COI sequences of *Ae. albopictus*, and data on the country of collection and references were extracted from the GenBank files. Only sequences covering a 624 bp section of the COI, not containing ambiguity characters and obvious sequencing errors, were selected for the DNA haplotype network analysis. The data originated from 26 publications and seven data sets published on GenBank only. For several data sets, the frequency of haplotypes had to be obtained from the respective publications. The data set contained 1680 individual records featuring 163 distinct COI haplotypes. To reduce the complexity of the network, all haplotypes recorded only once (n = 76) were removed, resulting in 86 haplotypes originating from 1603 individual records. After adding the data of the present study (adults obtained from the allotment garden, 49 individuals), the final alignment for the network analysis contained 1652 individual sequences. The DNA haplotype network analysis was performed using NETWORK v.10.0.0.0 (Fluxus Technology Ltd, Cambridge, UK) applying the default settings. The network was graphically arranged and provided with information on the geographic origin (United Nations Geoscheme) of the samples with Network Publisher v.2.1.2.5 (Fluxus Technology Ltd) and finalised using Adobe Illustrator CC v.2015.0.0 (Adobe Inc., San José, California, USA). In addition, pie charts showing the number of records per country were created for the three *Ae. albopictus* lineages detected in the present study using Microsoft Excel (Microsoft Office 365).

### 3 RESULTS

In the BGS trap at location A, 111 *Ae. albopictus* specimens (77 females and 34 males) were collected. In the two ovitraps at this site, 36 and 13 *Ae. albopictus* eggs were found, respectively. In the BGS trap at location B, no *Ae. albopictus* were captured. However, in one of the ovitraps at this site, 11 *Aedes japonicus* (Theobald, 1901) eggs were found. No eggs were found in the ovitraps at location C.

Our genetic analysis on *Ae. albopictus* revealed the presence of three different COI lineages in Vienna (Austria). Of the 49 samples from the allotment garden analysed by means of DNA barcoding of a 670 bp section of the COI, 26 individuals featured haplotype 1 (HPT1, MW457632), 22 haplotype 2 (HPT2, MW457633) and 1 haplotype 3 (HPT3, MW457634) (Figure 2). Apart from 26 *Ae. albopictus* individuals from Vienna, HPT1 was recorded only in single specimens in the United States (Zhong et al., 2013) and Canada (Giordano et al., 2019) (Figure 2). HPT2 represents one of the five most frequent *Ae. albopictus* lineages, and has been reported from Asia (n = 66), the Americas (n = 3) and Europe (n = 2) (Figure 3). Besides in the 22 *Ae. albopictus* individuals from Vienna, this lineage was found in Europe only in single specimens in the Netherlands (Van De Vossenberg et al., 2015) and Portugal (Zé-Zé et al., 2020) (Figure 3). HPT3 is by far the most frequent *Ae. albopictus* lineage worldwide and was detected in 647 specimens in Europe (n = 294), Asia (n = 217), the Americas (n = 127) and Hawai (n = 10) (Figure 2). This haplotype was recorded only in a single specimen from Vienna, but was recently also found in Tirol (Austria; Fuehrer et al., 2020). In Europe, HPT3 was also found in Spain (n = 166; Brustolin, 2016), Russia (n = 49; Fedorova & Shaikevich, unpublished), Italy (n = 31; Battaglia et al., 2016; Shaikevich & Talbalaghi, 2013; Zhong et al., 2013), Portugal (n = 22; Osório et al., 2018; Zé-Zé et al., 2020), the Netherlands (n = 20; Van De Vossenberg et al., 2015), Greece (n = 3; Battaglia et al., 2016; Bisia et al., 2019) and Albania (n = 2; Battaglia et al., 2016) (Figure 3).

In one of the ovitraps *Ae. albopictus* egg batches belonged to HPT1. In the second ovitraps haplotypes HPT1 and HPT2 were found. The adult captured during the mosquito surveillance program in Donaustadt was identified as HPT3.

### 4 DISCUSSION

With 1.9 million inhabitants, Vienna is the fifth largest city within the European Union. The many public parks and gardens, and ample suburban areas offer many potential breeding sites, facilitating the establishment of *Ae. albopictus* populations. In addition, favourable climatic conditions, caused by urban heat-island effects and artificial watering in large cities like Vienna, could increase the survival, breeding success and activity of arthropod vectors such as *Ae. albopictus* (Bradley & Altizer, 2007). Climate suitability models suggest that the climate in the area of Vienna would permit stable populations of *Ae. albopictus* to occur, especially if climate warming were to take place (Caminade et al., 2012; Fischer et al., 2011). An establishment of stable populations
FIGURE 2  Median-Joining DNA haplotype network based on a 624 bp section of the mitochondrial COI. The colour-coding of geographic regions follows the United nations geoscheme (see insert). Each coloured circle represents a unique COI haplotype/lineage. The size of the circles corresponds to the frequency, but the number is also indicated for all haplotypes featuring more than two records. Bars on branches indicate the number of substitutions between two haplotypes. Small white circles represent median vectors, which are hypothetical (often ancestral or unsampled) sequences required to connect existing haplotypes with maximum parsimony. The haplotypes detected in the present study in Vienna (Austria) are marked with asterisks.

FIGURE 3  Number of records per country for the three COI lineages of Ae. albopictus detected in the present study.
The establishment of stable populations of *Ae. albopictus* in Vienna would not only cause high economic costs (e.g. for vector control), but would also impair the quality of life in the city (Darbro et al., 2017; Halasa et al., 2014). The establishment of such potentially invasive mosquito species in Vienna would furthermore increase the threat of *Aedes*-borne arboviruses such as chikungunya, dengue and Zika. The detection of *Ae. albopictus* in the capital city of Austria by a Viennese citizen underlines the significance, scope and advantage of citizen science approaches. Inhabitants of this allotment garden (location A) were trained in trap setting and received training to differentiate between common native and expected potentially invasive mosquito species in 2018. Further immediate actions — comprising a close-meshed mosquito monitoring at potential introduction routes, raising awareness with regard to *Ae. albopictus* in Vienna, and providing information on how to prevent further spread and multiplication of this species — need to follow.

**ACKNOWLEDGEMENTS**

Financial support for barcoding was provided by the Austrian Federal Ministry of Education, Science and Research via an ABOL (Austrian barcode of Life: www.abol.ac.at) associated project within the framework of the ‘Hochschulraum-Strukturmittel’ Funds. The preliminary citizen science project was funded by the FWF – Top Citizen Science (TCS-35). The work was done within the framework of AIM-COST Action CA17108. Furthermore, we wish to thank all citizen scientists.

**DATA AVAILABILITY STATEMENT**

All sequences generated in this study are openly available at the NCBI database (https://www.ncbi.nlm.nih.gov) with the following accession numbers: MW457632–MW457634.

**ORCID**

Karin Bakran-Lebl https://orcid.org/0000-0001-8818-2483
Carina Zittra https://orcid.org/0000-0002-8963-6421
Josef Harl https://orcid.org/0000-0002-4915-3943
Bita Shafi-Barough https://orcid.org/0000-0002-0533-4777
David Ebner https://orcid.org/0000-0001-8300-9702
Francis Schaffner https://orcid.org/0000-0001-9166-7617
Hans-Peter Fuehrer https://orcid.org/0000-0002-4178-0133

**REFERENCES**

Battaglia, V., Gabrieli, P., Brandini, S., Capodiferro, M. R., Javier, P. A., Chen, X.-C., Achilli, A., Semino, O., Gomulski, L. M., Malaradza, A. R., Gasperi, G., Torroni, A., & Olivieri, A. (2016). The worldwide spread of the tiger mosquito as revealed by mitogenome haplogroup diversity. *Frontiers in Genetics*, 7, 206. https://doi.org/10.3389/fgene.2016.00208

Becker, N., Petrić, D., Zgomba, M., Boase, C., Madon, M., Dahl, C., & Kaiser, A. (2010). Mosquitoes and their control. Berlin: Springer.

Becker, N., Geier, M., Balczun, C., Bradersen, U., Huber, K., Kiel, E., Krüger, A., Lühken, R., Orendt, C., Plenge-Büning, A., Rose, A., Schaub, G. A., & Tannich, E. (2013). Repeated introduction of *Aedes albopictus* into Germany, July to October 2012. *Parasitology Research*, 112(4), 1787–1790. https://doi.org/10.1007/s00436-012-3230-1

Bisio, M., Jeffries, C. L., Lytra, I., Michaelakis, A., & Walker, T. (2019). The abundance and diversity of West Nile virus mosquito vectors in two Regional Units of Greece during the onset of the 2018 transmission season. *BioRxiv*, 735522. https://doi.org/10.1101/735522

Bradley, C. A., & Altizer, S. (2007). Urbanization and the ecology of wildlife diseases. *Trends in Ecology and Evolution*, 22(2), 95–102.

Brustolin, M. (2016). *Autochthonous and invasive mosquitoes of Catalonia as vectors of zoonotic arboviruses*. Universitat Autònoma de Barcelona.

Caminade, C., Medlock, J., Ducheyne, E., McIntyre, K. M., Leach, S., Baylis, M., & Morse, A. P. (2012). Suitability of European climate for the Asian tiger mosquito *Aedes albopictus*: Recent trends and future scenarios. *Journal of the Royal Society Interface*, 9(75), 2708–2717. https://doi.org/10.1098/rsif.2012.0138

Darbro, J., Halasa, Y., Montgomery, B., Muller, M., Shepard, D., Devine, G., & Mweebaze, P. (2017). An economic analysis of the threats posed by the establishment of *Aedes albopictus* in Brisbane, Queensland. *Ecolmical Economics*, 142, 203–213. https://doi.org/10.1016/j.ecolett.2017.06.015

ECDC. (2021a). *Aedes albopictus* – Factsheet for experts. Retrieved 28 January 2021, from https://www.ecdc.europa.eu/en/disease-vectors/factsheet-mosquito-factsheets/aedes-albopictus

ECDC. (2021b). *Mosquito maps*. Retrieved 29 January 2021, from https://www.ecdc.europa.eu/en/disease-vectors-surveillance-and-disease-data-mosquito-maps

Eritja, R., Palmer, J. R. B., Roiz, D., Sanpera-Calbet, I., & Bartumeus, F. (2017). Direct evidence of adult *Aedes albopictus* dispersal by car. *Scientific Reports*, 7(1), 1–15. https://doi.org/10.1038/s41598-017-12652-5

Fischer, D., Thomas, S. M., Niemitz, F., Reineking, B., & Beierkuhnlein, C. (2011). Projection of climatic suitability for *Aedes albopictus* Skuse (Culicidae) in Europe under climate change conditions. *Global and Planetary Change*, 78(1–2), 54–64. https://doi.org/10.1016/j.gloplacha.2011.05.008

Flacio, E., Engeler, L., Tonolla, M., & Müller, P. (2016). Spread and establishment of *Aedes albopictus* in southern Switzerland between 2003 and 2014: An analysis of oviposition data and weather conditions. *Parasites & Vectors*, 9(1), 304. https://doi.org/10.1186/s13071-016-1577-3

Fuehrer, H. P., Schoener, E., Weiler, S., Barogh, B. S., Zittra, C., & Walder, G. (2020). Monitoring of alien mosquitoes in Western Austria (Tyrol, Austria, 2018). *PLoS Neglected Tropical Diseases*, 14(6), e0008433. https://doi.org/10.1371/journal.pntd.0008433

Giordano, B., Gasparotto, A., Liang, P., Nelder, M., Russell, C., & Hunter, F. (2019). Discovery of an *Aedes* (Stegomyia) *albopictus* population and first records of *Aedes* (Stegomyia) *egypti* in Canada. *Medical and Veterinary Entomology*, 34, 10–16.

Halasa, Y. A., Shepard, D. S., Fonseca, D. M., Farajollahi, A., Healy, S., Gaugler, R., Bartlett-Healy, K., Strickman, D. A., & Clark, G. G. (2014). Quantifying the impact of mosquitoes on quality of life and enjoyment of yard and porch activities in new jersey. *PLoS ONE*, 9(3), e89221. https://doi.org/10.1371/journal.pone.0089221

Hall, T. A. (1999). BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, 41, 95–98.
Hebert, P. D. N., Penton, E. H., Burns, J. M., Janzen, D. H., & Hallwachs, W. (2004). Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly Astraptes fulgerator. Proceedings of the National Academy of Sciences of the United States of America, 101(41), 14812–14817. https://doi.org/10.1073/pnas.0406166101

Ibañez-Justicia, A. (2020). Pathways for introduction and dispersal of invasive Aedes mosquito species in Europe: A review. Journal of the European Mosquito Control Association, 38, 1–10.

Medlock, J., Hansford, K. M., Schaffner, F., Versteirt, V., Hendrickx, G., Zeller, H., & Bortel, W. V. (2012). A review of the invasive mosquitoes in Europe: Ecology, public health risks, and control options. Vector-Borne and Zoonotic Diseases, 12(6), 435–447.

Medlock, J., Hansford, K. M., Versteirt, V., Cull, B., Kampen, H., Fontenille, D., Hendrickx, G., Zeller, H., Van Bortel, W., & Schaffner, F. (2015). An entomological review of invasive mosquitoes in Europe. Bulletin of Entomological Research, 105(06), 637–663. https://doi.org/10.1017/S0007485315000103

Müller, P., Engeler, L., Vavassori, L., Suter, T., Guidi, V., Gschwind, M., Tonolla, M., & Flacio, E. (2020). Surveillance of invasive Aedes mosquitoes along Swiss traffic axes reveals different dispersal modes for Aedes albopictus and Ae. japonicus. PLoS Neglected Tropical Diseases, 14(9), e0008705. https://doi.org/10.1371/journal.pntd.0008705

Osório, H. C., Zé-Zé, L., Neto, M., Silva, S., Marques, F., Silva, A. S., & Alves, M. J. (2018). Detection of the invasive mosquito species Aedes (Stegomyia) albopictus (Diptera: Culicidae) in Portugal. International Journal of Environmental Research and Public Health, 15(4), 820. https://doi.org/10.3390/ijerph15040820

Schoener, E., Zittra, C., Weiss, S., Walder, G., Barogh, B. S., Weiler, S., & Fuehrer, H. P. (2019). Monitoring of alien mosquitoes of the genus Aedes (Diptera: Culicidae) in Austria. Parasitology Research. https://doi.org/10.1007/s00436-019-06287-w

Scholte, E. J., & Schaffner, F. (2007). Waiting for the tiger - establishment and spread of Aedes albopictus mosquito in Europe. In W. Takken & B. G. J. Knols (Eds.), Emerging pests and vector-borne diseases in Europe. volume 1: Ecology and control of vector-borne diseases (pp. 241–260). Wageningen: Wageningen Academic.

Seidel, B., Duh, D., Nowotny, N., & Allerberger, F. (2012). Erstnachweis der Stechmücken Aedes (Ochlerotatus) japonicus japonicus (Theobald, 1901) in Österreich und Slowenien in 2011 und für Aedes (Stegomyia) albopictus (Skuse, 1895) in Österreich 2012 (Diptera: Culicidae). Entomologische Zeitschrift – Stuttgart, 112(5), 223–226.

Severini, F., Di Luca, M., Toma, L., & Romi, R. (2008). Aedes albopictus in Rome: Results and perspectives after 10 years of monitoring. Parasitologia, 50(1–2), 121–123.

Shaikevich, E., & Talbalaghi, A. (2013). Molecular Characterization of the Asian Tiger Mosquito Aedes albopictus (Skuse) (Diptera: Culicidae) in Northern Italy. ISRN Entomology, 2013, 157426. https://doi.org/10.1155/2013/157426

Van De Vossenberg, B. T. L. H., Ibáñez-Justicia, A., Metz-Verschure, E., Van Veen, E. J., Bruil-Dieters, M. L., & Scholte, E. J. (2015). Real-time PCR tests in Dutch Exotic Mosquito Surveys: Implementation of Aedes aegypti and Aedes albopictus identification tests, and the development of tests for the identification of Aedes atropalpus and Aedes japonicus japonicus. Journal of Medical Entomology, 52(3), 336–350. https://doi.org/10.1093/jme/tjv020

Zé-Zé, L., Borges, V., Osório, H. C., Machado, J., Gomes, J. P., & Alves, M. J. (2020). Mitogenome diversity of Aedes (Stegomyia) albopictus: Detection of multiple introduction events in Portugal and potential within-country dispersal. BioRxiv, 2020.02.12.945741. https://doi.org/10.1101/2020.02.12.945741

Zhong, D., Lo, E., Hu, R., Metzger, M. E., Cummins, R., Bonizzoni, M., Fujioka, K. K., Sorvillo, T. E., Kluh, S., Healy, S. P., Fredregill, C., Kramer, V. L., Chen, X., & Yan, G. (2013). Genetic analysis of invasive Aedes albopictus populations in Los Angeles County, California and its potential public health impact. PLOS ONE, 8(7), e68586.

How to cite this article: Bakran-Lebl, K., Zittra, C., Harl, J., Shahi-Barogh, B., Grätzl, A., Ebmer, D., Schaffner, F., & Fuehrer, H.-P. (2021). Arrival of the Asian tiger mosquito, Aedes albopictus (Skuse, 1895) in Vienna, Austria and initial monitoring activities. Transboundary and Emerging Diseases, 1–6. https://doi.org/10.1111/tbed.14169