StartClim2017.D

Monitoring of alien mosquitoes of the genus Aedes in Austria

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Inhaltsverzeichnis

Kurzfassung

Im Sommer 2017 wurde ein Projekt zur Überwachung von Eiablagen von neobiotischen Stechmücken der Gattung *Aedes* in fünf Bundesländern Österreichs - Kärnten, Wien, Niederösterreich, Steiermark und Burgenland - durchgeführt. Dafür wurden so genannte „Ovitraps", die unter anderem von mitwirkenden Citizen Scientists in deren Gärten aufgestellt wurden, verwendet.

Eier der asiatischen Tigermücke (*Ae. albopictus*), einem bedeutenden Überträger von Arboviren, wurden in keinem der teilnehmenden Bundesländer gefunden, während die japanische Buschmücke (*Ae. japonicus*) in Niederösterreich, der Steiermark und dem Burgenland nachgewiesen wurde. In Wien und Kärnten waren alle Fallen negativ für Ablagen von *Aedes*-Eiern.

Mit dem vorliegenden Projekt zeigen wir den Nutzen von einfachen Überwachungsmaßnahmen von Stechmücken unter der Verwendung von Ovitraps durch Citizen Scientists.

Abstract

In summer 2017 a monitoring project for oviposition of alien mosquito species belonging to the genus *Aedes* was performed in five Austrian districts, Carnithia, Vienna, Lower Austria, Styria and Burgenland. Instrumental for this project were so called "ovitraps" which were placed in parks and private gardens by participating Citizen Scientists.

Eggs of the Asian tiger mosquito (*Ae. albopictus*), an important vector for arboviruses, were found in none of the examined provinces, while eggs of the Japanese bush mosquito (*Ae. japonicus*) were found in Lower Austria, Styria and Burgenland. In Vienna and Carnithia, all traps were negative for *Aedes* eggs.

With this project, we demonstrated the usefulness of citizen scientists for mosquito surveillance using ovitraps.

D-1 Introduction

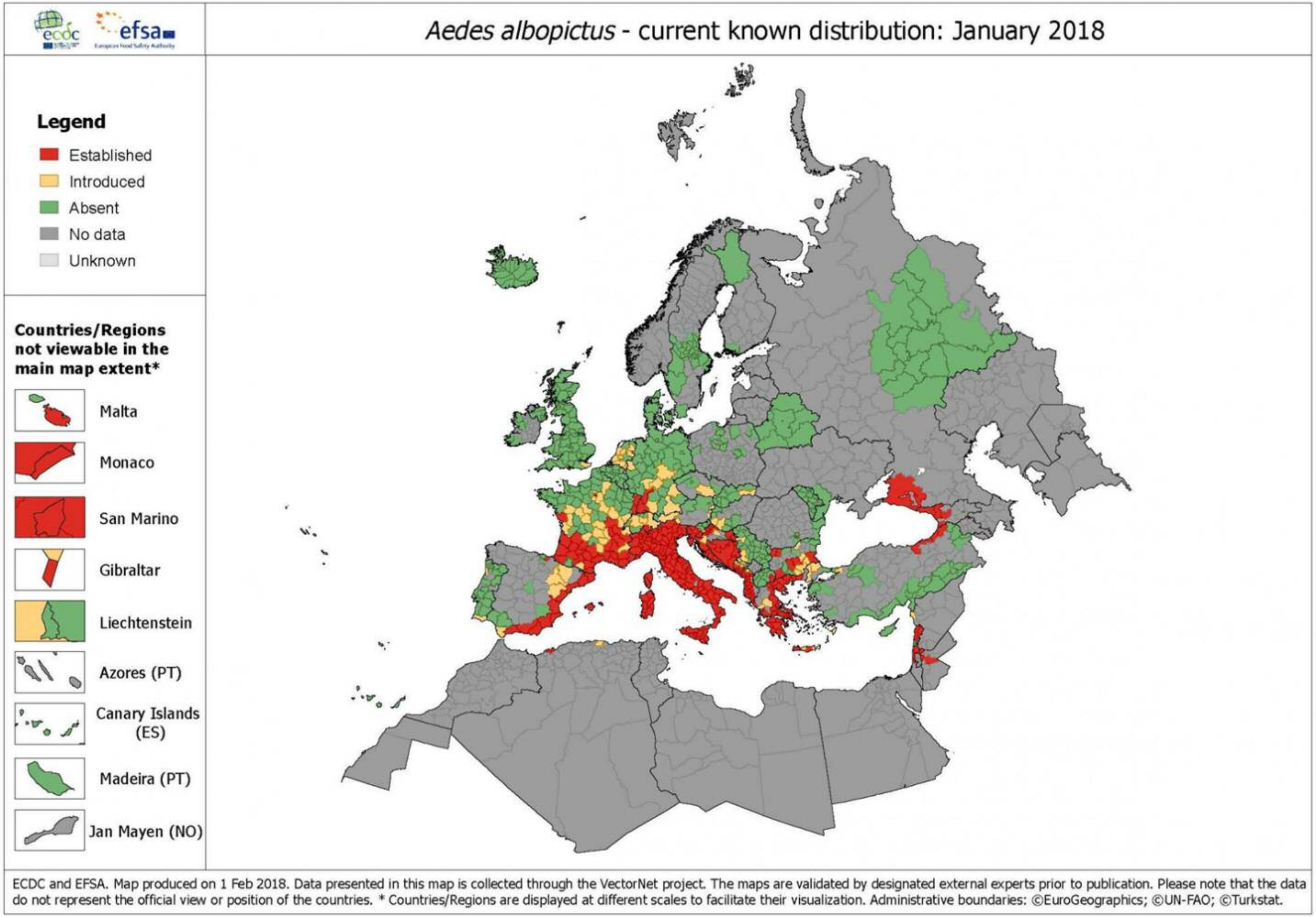
49 indigenous species of the family Culicidae from eight genera (*Aedes*, *Anopheles*, *Culex*, *Coquillettidia*, *Culiseta*, *Ochlerotatus*, *Orthopodomyia* and *Uranotaenia*) have been specified in Austria (Zittra et al. 2015, Zittra et al. 2017). The establishment of the potential invasive species *Aedes albopictus* (Asian tiger mosquito) and *Aedes japonicus japonicus* (Japanese bush mosquito) in Austria has been in dispute until recently (Zittra et al. 2015). Three additional non-native species, *Anopheles hyrcanus*, *Orthopodomyia pulcripalpis* and *Culiseta longiareolata*, have been reported (e.g. Zittra et al., 2017, Zittra et al., 2015).

Alien mosquitoes are expanding their range in Europe, and two species, *Aedes albopictus* (Fig. D- 1) and *Aedes japonicus japonicus* (Fig. D- 2), have been known for their invasive behaviour.

Fig. D- 1: *Aedes albopictus*, the Asian tiger mosquito (male); Source: https://ecdc.europa.eu/en/disease-vectors/facts/mosquito-factsheets/aedes-albopictus

Fig. D- 2: *Aedes japonicus*, the Japanese bush mosquito; Source: https://ecdc.europa.eu/en/disease-vectors/facts/mosquito-factsheets/aedes-japonicus

Whereas *Ae. albopictus* is currently restricted to Southern Europe, it is expected to expand its range with ongoing climate change (Cunze et al. 2016), *Ae. japonicus* is from a temperate climate zone and while its range will expand into Northern and temperate regions of Europe, it is not able to adapt to warmer climates (Cunze et al. 2016). Both species originate from Asia and have increased their range in Europe over the past decade (Medlock et al. 2015, Cunze et al. 2016) (Figs. D- 3+4). This is of importance, because the Asian tiger mosquito *Ae. albopictus* is known for its high ecological and climatic adaptability and is now found as an invasive species on all continents except Antarctica (Bonizzoni et al. 2013). It is adaptable enough to adjust to more temperate climates (Goubert et al. 2017) and since its introduction into Germany, it was already able to reproduce in high numbers at certain sites in 2015 (Becker et al. 2017). The Asian tiger mosquito is of public health concern since it is a vector of different arboviruses (Paupy et al. 2009, Schaffner et al. 2013) like Dengue (Rezza 2012, Ferreira-de-Lima and Lima-Camara 2018) and Chikungunya virus (Poletti et al. 2011, Carrieri et al. 2012). While *Ae. japonicus* appears to be of lesser public health importance, it has been non-the-less shown to be a potential vector for West- Nile- virus in laboratory experiments (Wagner et al. 2018).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Canary Islands (ES)
Madeira (PT)
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ECDC and EFSA. Map produced on 1 Feb 2018. Data presented in this map is collected through the VectorNet project. The maps are validated by designated external experts prior to publication. Please note that the data do not represent the official view or position of the countries. * Countries/Regions are displayed at different scales to facilitate their visualization. Administrative boundaries: ©EuroGeographics; ©UN-FAO; ©Turkstat.

Fig. D- 3*: Aedes albopictus*- current known distribution: January 2018; https://ecdc.europa.eu/en/publications-data/aedes-albopictus-current-known-distribution-january-2018

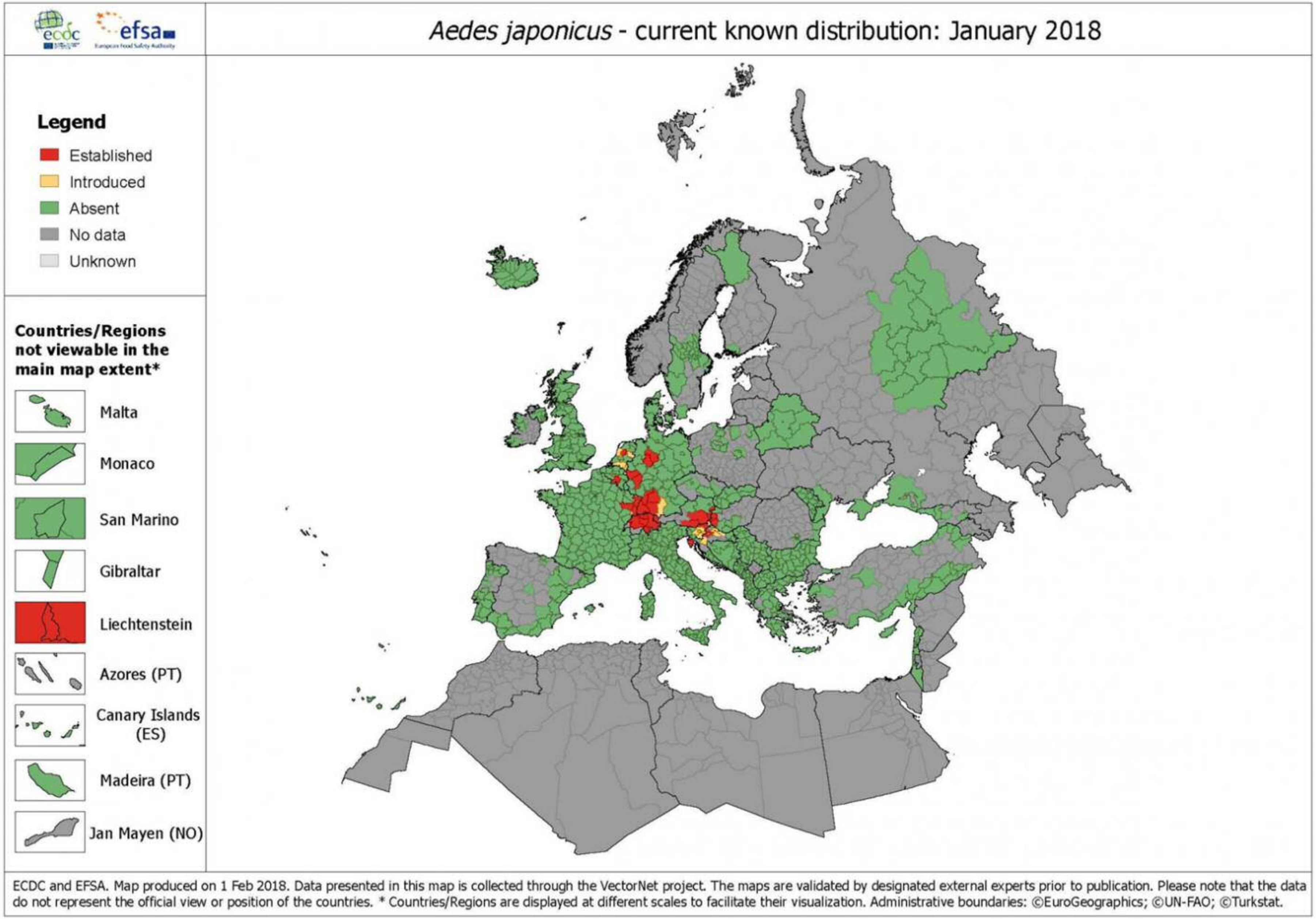
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Fig. D- 4: *Aedes japonicus*- current known distribution: January 2018; https://ecdc.europa.eu/en/publications-data/aedes-japonicus-current-known-distribution-january-2018

For the surveillance of invasive *Aedes* mosquito species, ovitraps have been frequently used (Carrieri et al. 2012, Velo et al. 2016, Baldacchino et al. 2017, Di Luca et al. 2017, Manica et al. 2017). For this method, small water containers are set up at sampling sites and some form of substrate is provided for oviposition of female mosquitoes, like wooden paddles or germination paper (Velo et al. 2016, Manica et al. 2017). The ovitraps are then collected and checked for mosquito eggs on the provided substrate. It could be demonstrated that this is a reliable method for the surveillance of *Ae. albopictus* and a good predictor for adult biting mosquitoes (Manica et al. 2017).

In summer 2017, a surveillance project using ovitraps, which were set up in five federal states of Austria, was performed, to determine the presence and distribution of the alien mosquito species *Ae. albopictus* and *Ae. japonicus*.

D-2 Material and Methods

In summer 2017, surveillance of mosquito oviposition, using ovitraps, was performed in five federal states of Austria, Carinthia, Vienna, Lower Austria, Styria and Burgenland: 14 sites in Carinthia (Fig. D- 5), 10 in Vienna (Fig. D- 6), 10 in Lower Austria (Fig. D- 7), 17 in Styria (Fig. D- 8) and 7 in Burgenland (Fig. D- 9).

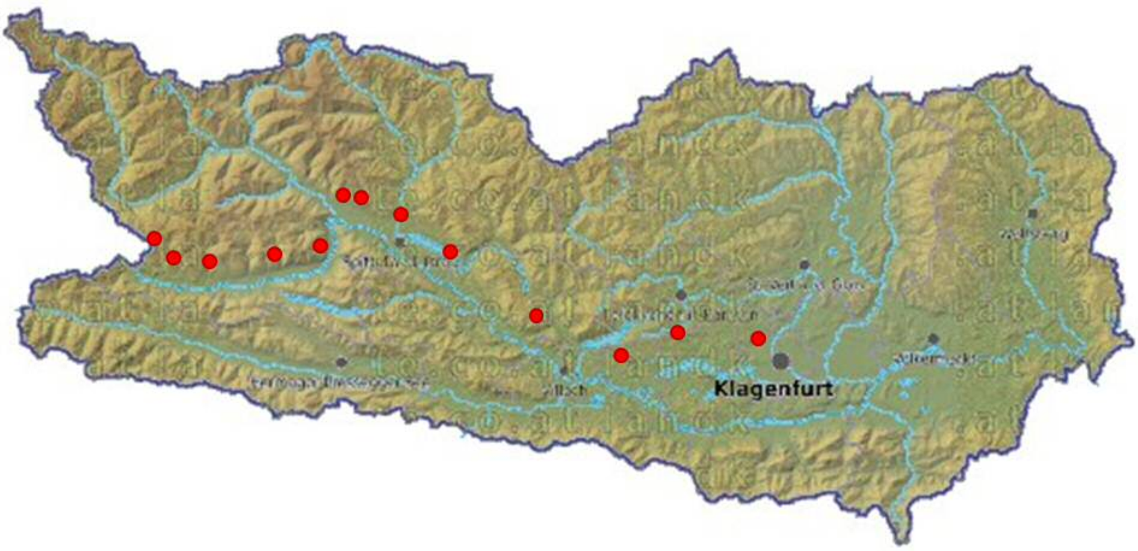

Fig. D- 5: Ovitrap sampling sites in Carinthia

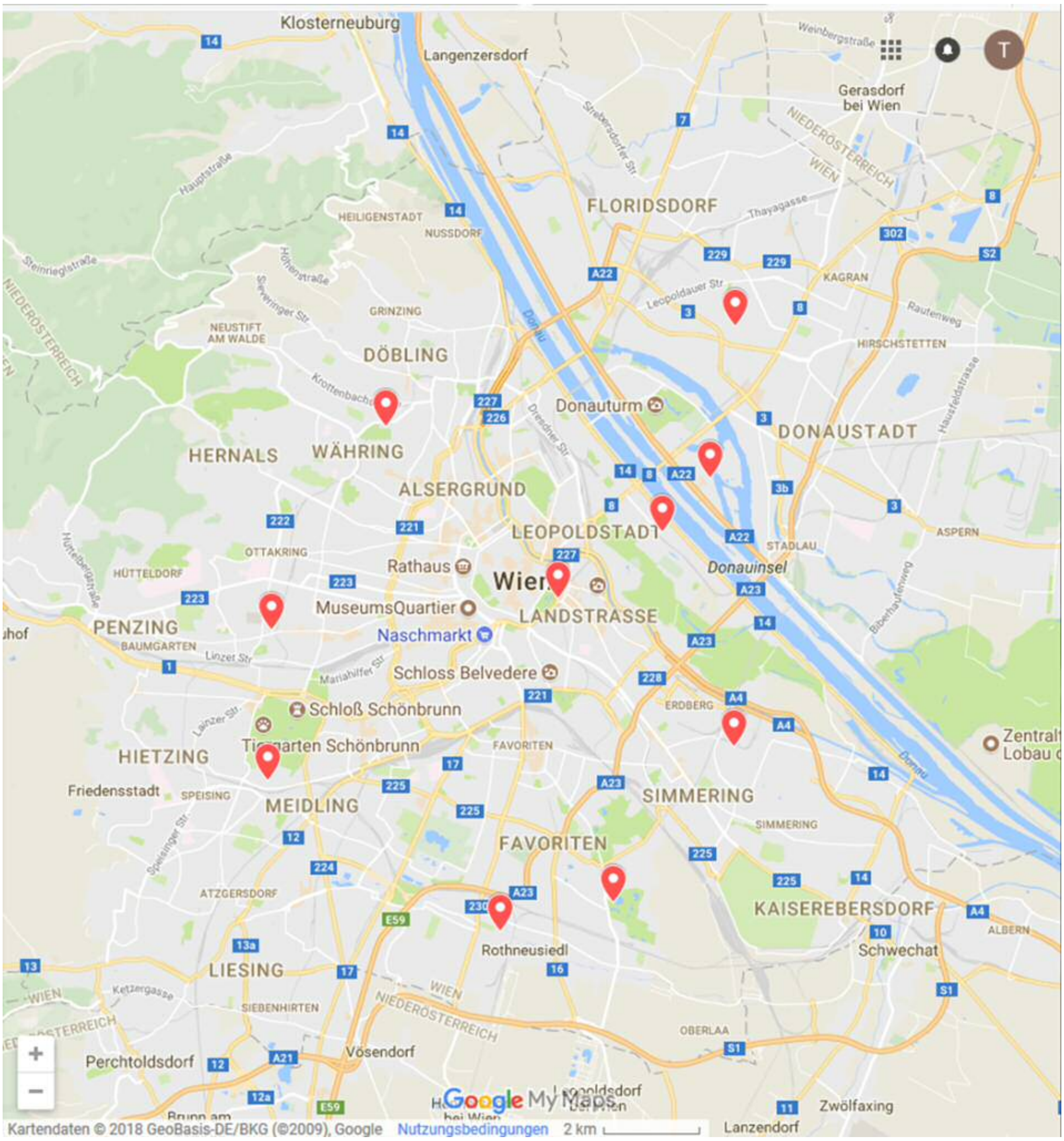

Fig. D- 6: Ovitrap sampling sites in Vienna

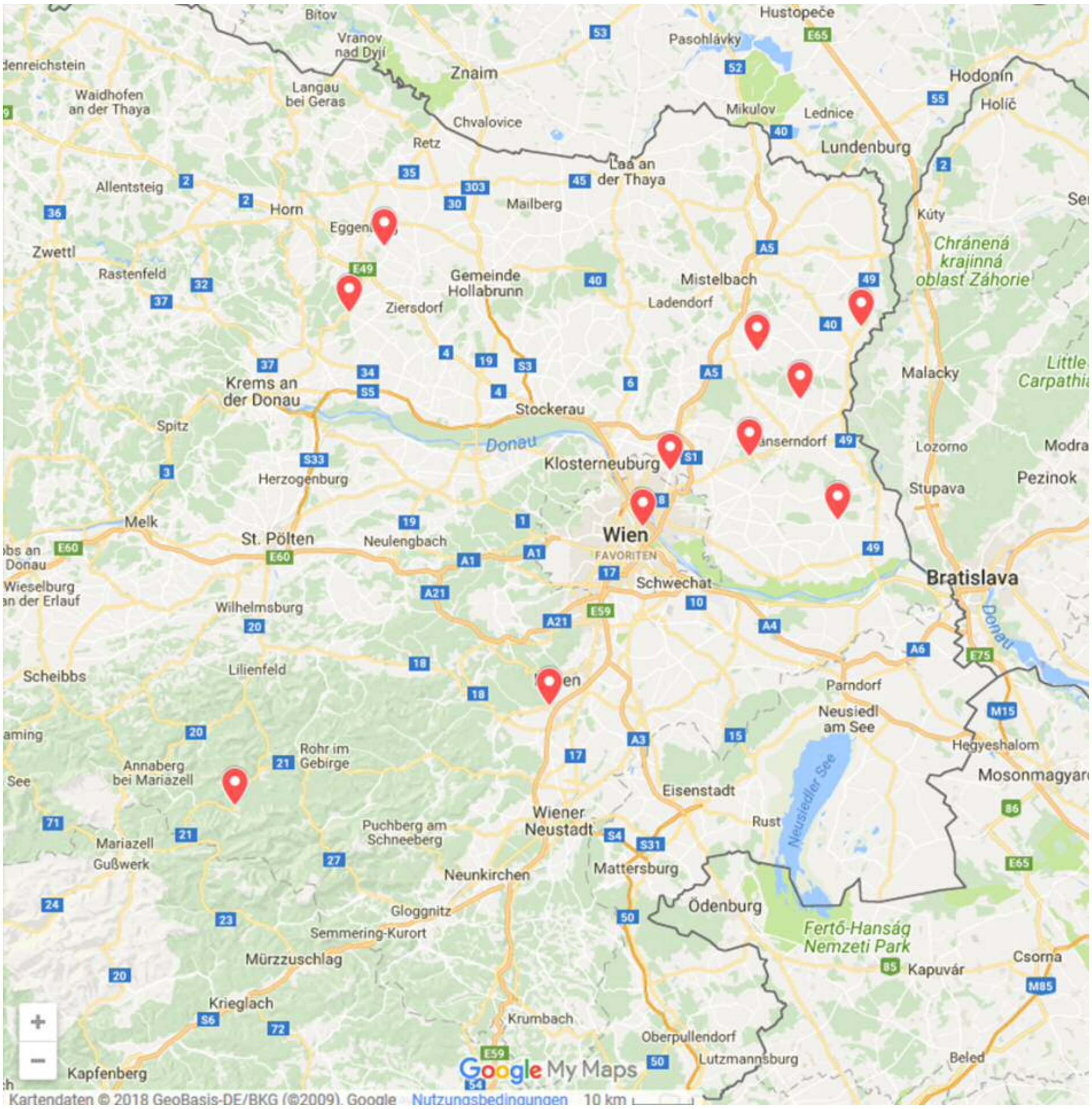

Fig. D- 7: Ovitrap sampling sites Lower Austria

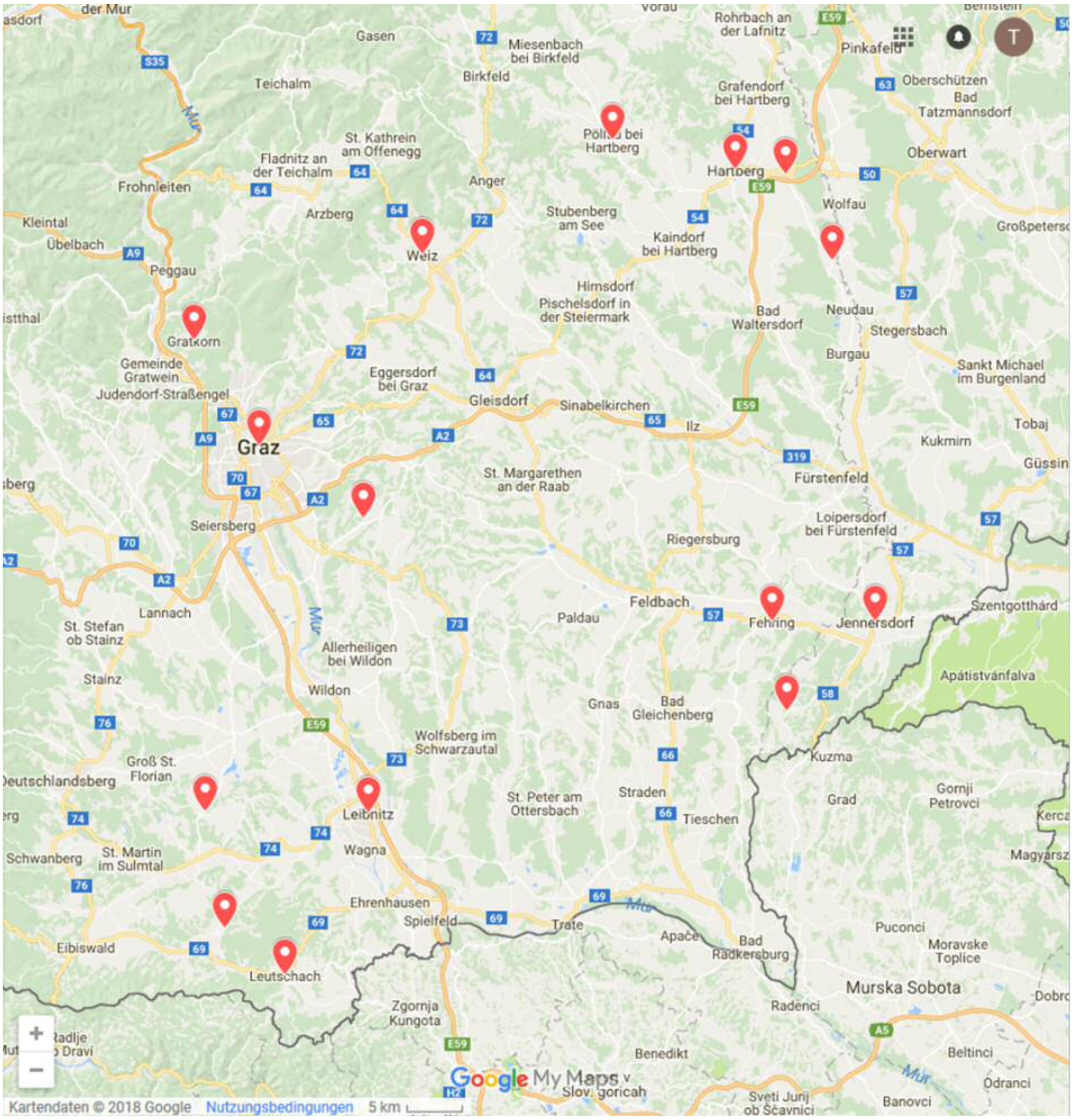

Fig. D- 8: Ovitrap sampling sites in Styria

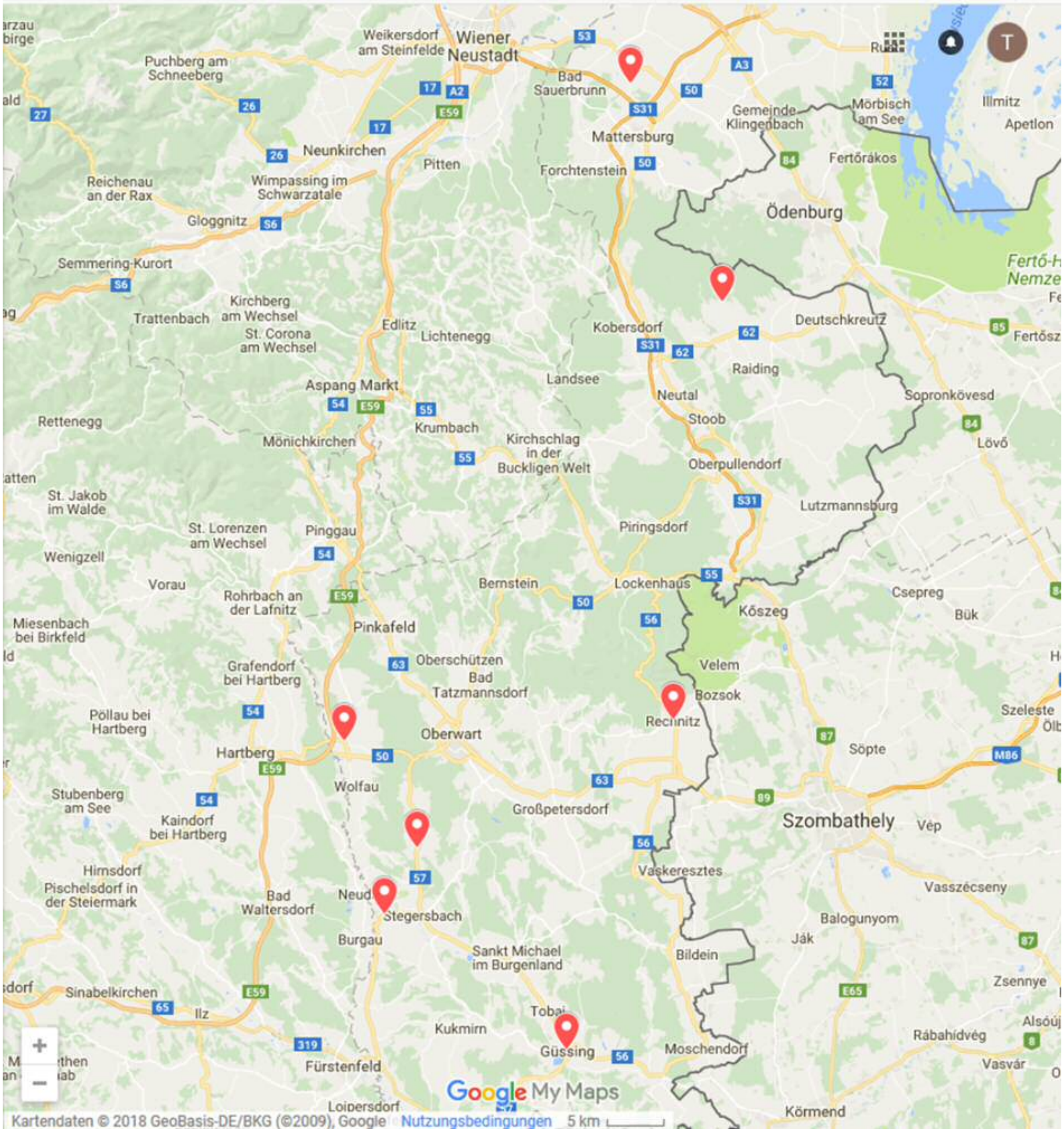

Fig. D- 9: Ovitrap sampling sites in Burgenland

The sampling started at the beginning of June in Carinthia and in July at all other sites and ended at all sites in the end of October. The traps in Carinthia were sampled weekly, whereas the other sites were sampled once a month for a week.

The method of using ovitraps has been utilized widely for surveillance of invasive *Aedes* species and is, for example, described in (Manica et al. 2017). We set up two 500 ml black conical cubs per site, filled with approximately 400 ml tap water, with wooden paddles (2x20 cm) inserted into the water as a substrate for oviposition by the mosquito females (Figs. D-10+11). All ovitraps are labelled (see Supplement D- 1) for numbering and prevention of interference with the traps. Since we partially relied on the help of citizen scientists, two information sheets were handed out with instructions on how to use the ovitraps (Supplements D 2+3).

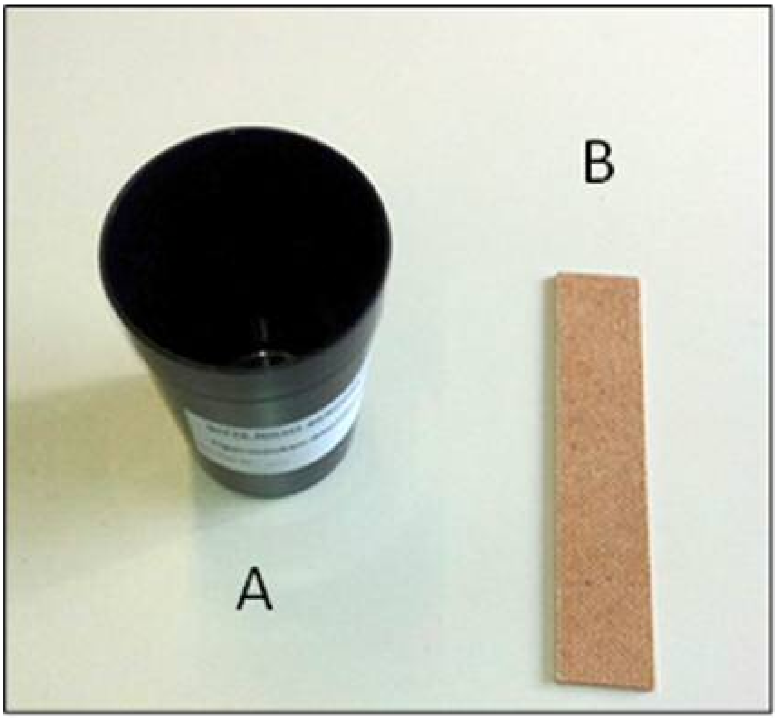

Fig. D- 10: The Ovitrap consists of (A) a black plastic cup (300 ml) and (B) a wooden paddle for oviposition

Fig. D- 11: The Ovitrap is filled to about ¾ (white line) with fresh tap water and the wooden paddle is inserted with the rough side facing upward

After collection, the wooden paddles were examined under a dissection microscope for *Aedes* eggs and present eggs were counted. In the provinces Vienna, Lower Austria, Styria and Burgenland, the eggs on positive paddles were submitted to DNA extraction and PCR. DNA was extracted from individual eggs using the Qiagen DNeasy Blood and Tissue kit (Qiagen/ Germany). To each sample, 180 µl buffer ATL, 20 µl proteinase K and two ceramic beads (Precellys Ceramic Beads, Peqlab Biotechnologie GmbH) were added and homogenized in a TissueLyser II (Qiagen, Germany). The samples were than incubated at 56°C overnight and processed according to specifications. To identify mosquito species, a PCR was performed using the primers LCO1490 and HCO2198 as described in (Folmer, Black et al. 1994). Reaction products were commercially sequenced at LGC Genomics GmbH, Germany, and resulting sequences compared for similarity to sequences available on the GenBank® database (http://www.ncbi.nlm.nih.gov/BLAST).

D-3 Results

During this project, eggs of alien mosquito species were found, but their presence varied by state and month. No eggs of *Ae. albopictus* were identified, while *Ae. japonicus* was found in Lower Austria, Styria and Burgenland (Table D- 1). In Vienna and Carinthia, all ovitraps were negative for *Aedes* eggs. In Lower Austria, one site was positive for eggs of *Ae. japonicus japonicus* in July and August 2017, in Styria, two sites were positive for *Ae. japonicus japonicus* eggs in August and one site in September (Figs. D- 12+13). *Ae. japonicus japonicus* was also identified in Burgenland, where two sites were positive in August (Table D- 1).

Table D- 1: Eggs of *Aedes japonicus* collected in ovitraps in summer 2017 in the Austrian federal states Lower Austria, Styria and Burgenland

Month	calendar week	State	Postal Code	Town	Species found	Number of eggs present
July	29	Lower Austria	3195	Kernhof	*Aedes japonicus japonicus*	9
August	34	Lower Austria	3195	Kernhof	*Aedes japonicus japonicus*	12
August	31	Styria	8430	Leibnitz	*Aedes japonicus japonicus*	29
August	32	Styria	8453	St.Johann im Saggautal	*Aedes japonicus japonicus*	33
August	35	Styria	8444	St. Andrä-Höch	*Aedes japonicus japonicus*	52
August	35	Burgenland	7471	Rechnitz	*Aedes japonicus japonicus*	12
September	37	Burgenland	7032	Sigleß	*Aedes japonicus japonicus*	16

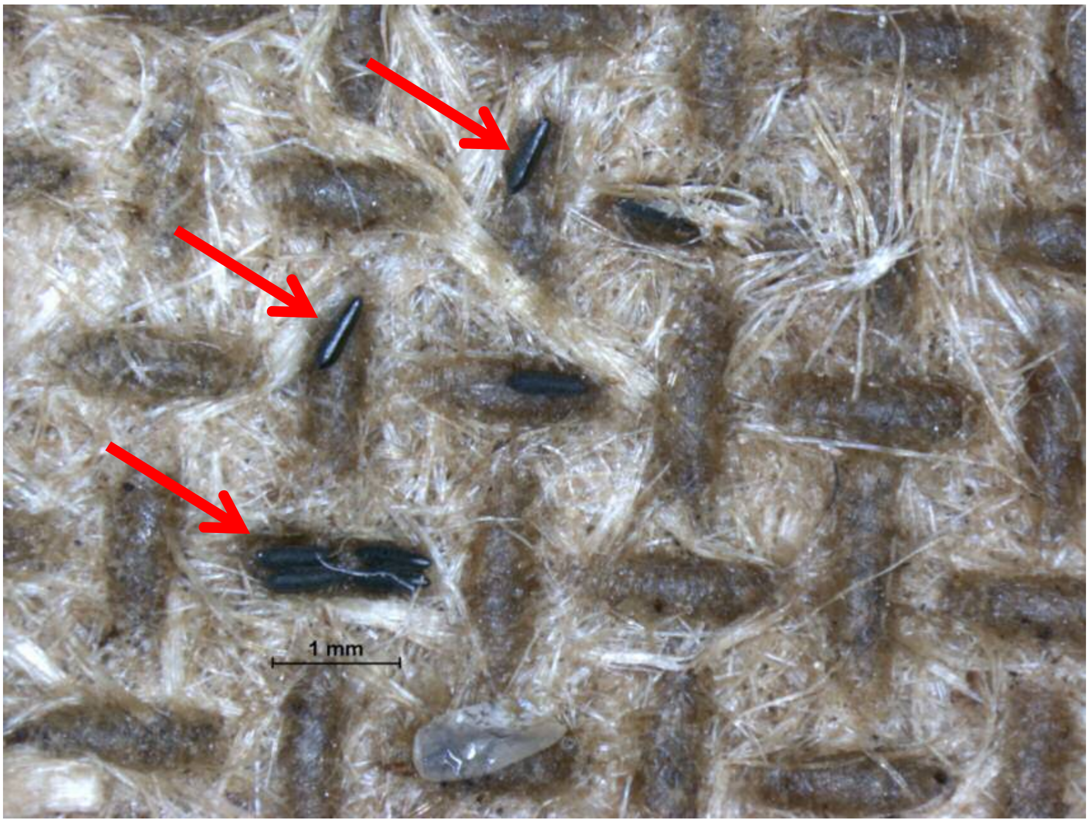

Fig. D- 12: *Aedes japonicus* eggs (arrows) on a wooden paddle from an ovitrap in Lower Austria.

Fig. D- 13: *Aedes japonicus* egg on a wooden paddle from an ovitrap in Lower Austria.

D-4 Discussion

Eggs of the alien mosquito species *Ae. japonicus* were found in three of the examined federal states of Austria during the late summer months of 2017, however, no eggs the Asian tiger mosquito *Ae. albopictus* were detected. The Asian tiger mosquito, which is native to South East Asia, is currently restricted mainly to Southern Europe, but due to ongoing climate change is expected to expand its range further northwards (Caminade et al. 2012, Medlock et al. 2015, Cunze et al. 2016). It is established in Italy and has even reached its southernmost Mediterranean islands (Di Luca et al. 2017) and likely played a role in the Italian outbreak of Chikungunya virus in 2007 (Poletti et al. 2011, Carrieri et al. 2012). *Aedes albopictus* is now also found in Switzerland (Flacio et al. 2016) and Germany (Becker et al. 2017), countries neighbouring of Austria. In future years, it is likely that *Ae. albopictus* will disperse further along travel routes from the South and future surveillance in the following years will show if the species is able to establish a self-sustaining population.

Like *Ae. albopictus*, *Ae. japonicus* is expanding its range in Europe. During this study, eggs of the species were found in three Austrian states. This finding is not surprising, since *Ae. japonicus* has been spreading in Austria for the past years and has been present in Carinthia, Burgenland, Vorarlberg and Tyrol (Seidel et al. 2016). It is also found in the neighbouring countries Germany (Kampen et al. 2016) and Hungary (Seidel et al. 2016) and has also been detected in Italy (Seidel et al. 2016).

D-5 Conclusion

With this project, we demonstrated the usefulness of citizen science for mosquito surveillance with ovitraps. The alien species *Ae. japonicus* continues to expand its range in Austria as we could show during this study. Although no *Ae. albopictus* eggs were detected, there is evidence (G. Walder pers. comm.) that this alien species is continuously introduced into Austria along main traffic routes from Italy, where this mosquito is well established. The risk of establishment of the Asian tiger mosquito in the Lower Inn Valley is therefore high and we recommend informing the general public about preventive measures to hinder and delay this development. A combination of ovitraps and adult female mosquito sampling (carbon dioxide baited traps) is recommended (ovitraps = collection of eggs vs. BioGents = collection of blood seeking mosquitoes).

Overall Citizen Science has shown to be an adequate tool for mosquito research as long scientific supervision is given adequately (incl. information about results). Ovitraps are easy to handle and are so perfectly suited for citizen science. However, previous studies have also shown that permanent sampling sites (collection once monthly from April to October) for adult female mosquitoes using carbon dioxide baited traps (BG Sentinel) can also be handled by citizen scientists but the level of scientific support/supervision is more intense in comparison to ovitraps.

Currently the monitoring systems in Austria vary between the provinces (Bundesland; e.g. monitoring by AGES and other research facilities). In Austria the provinces are those responsible that mosquito monitoring is performed. It is strongly recommended to unify and coordinate mosquito management in Austria (e.g. collaboration of provinces responsible for mosquito monitoring) in accordance with the mosquito monitoring guidelines of the ECDC. This will not only lead to an adequate monitoring scheme for potential invasive mosquitoes (and mosquito borne diseases) but will also make prevention strategies easier (e.g. in case of an increase of WNV infections after heat periods or flooding events). Moreover it would make it easier to compare results of various teams. In general Austrian mosquito monitoring data should be (and in general is) provided to the ECDC (e.g. for mosquito maps; https://ecdc.europa.eu/en/disease-vectors/surveillance-and-disease-data/mosquito-maps). Furthermore an EU-wide COST action (*Aedes* invasive mosquitoes -CA17108) tries to solve following problem: “Trans-boundary risks require effective surveillance, risk assessment, and vector control, with efficient dissemination of information and guidance to stakeholders, requiring collaboration between the normative, research, public health, commercial and civil society sectors at international, national and local scales. This is not happening. Despite the range of institutional guidelines available, current mitigation activities are largely uncoordinated, and implemented piecemeal nationally or locally, reducing cost-effectiveness and impact”.

To conclude, following recommendations are proposed:

- Nationwide coordination of vector sampling (incl. providing data to a database – ECDC and/or AGES-datebase) plus molecular based databases
- more intense (e.g. monthly) and expanded (e.g. district level) monitoring scheme for alien mosquitoes and West Nile Virus vectors
- inclusion of citizen scientists (in close collaboration - supervised by scientists) to set up mosquito traps (e.g. ovitraps for invasive *Aedes* mosquito egg sampling)

Overall more intense monitoring and coordinators result in an increase of costs and therefor more funding is required. However, the inclusion of scientist supervised citizen scientists reduce the need of additional financial support but our recent studies have shown that experienced personnel is needed to supervise citizen scientists.

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Figures and Tables

Figures

Tables