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Range expansion of the alien mosquito species *Aedes japonicus* (Theobald, 1901) (Diptera: Culicidae) from 2014–2019 in Burgenland, Austria.

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Abstract: Range expansion of the alien mosquito species *Aedes japonicus* (Theobald, 1901) (Diptera: Culicidae) from 2014 – 2019 in Burgenland, Austria. In the last 30 years, various *Aedes* species were introduced outside their native range. The potentially invasive mosquito species *Ae. japonicus* was recorded in Europe in 2000 and was found for the first time in Austria in 2011. Within the scope of a mosquito-monitoring program (2014–2019) the range expansion of *Ae. japonicus* in the province of Burgenland (Austria) was investigated. Throughout the sampling period 130 female *Ae. japonicus* were collected. The first specimens were captured in 2014 in the southernmost sampling sites. In the following years this species increased its range northwards, on average covering 13.6 km/year (sd=20.87). In 2019, its northernmost distribution was in the district Mattersburg, which is located 67.9 km north of the northernmost distribution in 2014 (Güssing). Adults of *Ae. japonicus* were captured from May to November, with the highest numbers trapped in July and August. The continuative range expansion of this species from the south to the north suggests that this process is driven by natural factors like active dispersal or wind. We found *Ae. japonicus* not to be very abundant but common in Burgenland. At present, there is no proof that *Ae. japonicus* is invasive in Austria. More in-depth and long-term investigations of *Ae. japonicus* are necessary to investigate their interactions with autochthonous species to assess whether this species can become invasive.

Keywords: mosquitoes, *Aedes japonicus*, Asian bush mosquito, Asian rock hole mosquito, dispersal, invasive species, monitoring

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Introduction

In the last 30 years five aedine mosquito species, *Ae. albopictus*, *Ae. aegypti*, *Ae. japonicus*, *Ae. atropalpus and Ae. koreicus* were introduced to areas outside their native range (Schaffner et al. 2013). At present, *Ae. albopictus*, *Ae. japonicus* and *Ae. koreicus* are an in the focus of public health concerns in Austria (Schoener et al. 2019, Fuehrer et al. 2020). Accidental introduction and passive spreading of those alien species has been facilitated by global cargo distribution with global trade of used tyres and lucky bamboo as the main means of transportation (Medlock et al. 2012). Recently, the introduction of alien mosquitoes via air-crafts was demonstrated (Ibáñez-Justicia et al. 2020).

Populations of introduced mosquito species can establish if suitable climatic conditions are found in these areas (Medlock et al. 2015, Cunze et al. 2016). Non-native mosquito species can become invasive if their introduction and/or spread threaten biological diversity (Lucy et al. 2016) and therefore may pose a potential danger in causing environmental or economic damage after establishing stable populations. In the worst case the arrival of alien species can be followed by disease outbreaks caused by transmitted pathogens (e.g. Chikungunya, Dengue, and Zika viruses) which autochthonous mosquitoes are not able (or not known to be able) to transmit (Juliano & Lounibos 2005).

The Asian bush mosquito or Asian rock pool mosquito, *Aedes japonicus*, (Theobald, 1901) (Fig. 1) is native to Korea, Japan, Taiwan as well as to southern China and south eastern Russia (Kaufman & Fonseca 2014). *Aedes japonicus* comprises four allopatric subspecies: *Ae. j. japonicus*, *Ae. j. shintienensis* Tsai & Lien, *Ae. j. yaeyamensis* Tanaka, Mizusawa & Saugstad, and *Ae. j. amamiensis* Tanaka, Mizusawa & Saugstad (Kaufman & Fonseca 2014). However, only *Aedes j. japonicus* is known to disperse successfully outside its native range (Kampen & Werner 2014). Its adaptation to the temperate climate zone, by producing cold- and desiccation-resistant eggs (Reuss et al., 2018), and the accompanying seasonal temperature fluctuations in the temperate climate is probably responsible for the successful expansion.

Aedes japonicus was unintentionally introduced to Europe possibly by global trade of used tires, and was initially documented in Europe in 2000 in Normandy (Orne), in the north of France (Medlock et al. 2012, Medlock et al. 2015), where it was not able to establish (Schaffner et al. 2009). The species has since been detected in Belgium (in 2002), in Switzerland (in 2008), and in Germany in (2011; Koban et al. 2019). In Austria, the Asian bush mosquito was first mentioned in 2011, in Styria (Seidel et al. 2012). Till now, this species has further been reported for Slovenia (Seidel et al. 2012), Italy (Montarsi et al. 2019), Hungary (Sáringer-Kenyeres et al. 2020), Croatia (Klobu-ar et al. 2019), the Netherlands (Abbo et al. 2020) Spain (Eritja et al. 2019) and Luxembourg (Schaffner & Ries 2019).

In its native range, female *Ae. japonicus* choose a wide variety of shaded natural or artificial containers filled with water rich in organic matter as oviposition habitats, preferring rock holes (Tanaka et al. 1979). Further, adults were described to inhabit forested areas, and to be absent in metropolitan areas (Tanaka et al., 1979). Investigations of macrohabitats yielded inconsistent results: On the one hand *Ae. japonicus* is described as an urban species (cf. Medlock et al 2012), but on the others characterizes adult *Ae. japonicus* as a rural, mainly sylvatic mosquito species, using suburban and urban areas for egg-laying, when a proximity and ecological contiguity is given to forested areas (cf. Juliano & Lounibos 2005, Balestrino et al. 2016). Outside its native range preferred breeding sites of *Ae. japonicus* are small, often artificial, vessels like e.g. cemetery flower pots or vases, buckets, ash trays, bird baths, water fountains, watering cans, paddling pools or catch basins (Schaffner et al. 2009, Sáringer-Kenyeres et al. 2020).

Fig. 1: Female Asian bush mosquito (*Ae. japonicus*). It is characterised by: 3 white rings on the hind legs; the tergites are dark with lateral white spots; the thorax is brown with golden stripes; palps and proboscis are dark. Foto: © Gernot Kunz.

Mammals including humans are preferred hosts (Apperson et al. 2004, Medlock et al. 2012, Goodman et al. 2018). Experiments under laboratory conditions indicate that *Ae. japonicus* may be a vector of Japanese encephalitis virus (Takashima et al. 1989), West Nile virus (Sardelis & Turell 2001) and dengue virus (Schaffner et al. 2011). The species may also play a role in the transmission of Chikungunya virus (Martinet et al. 2019). Recently, Usutu virus has been detected in samples of this species captured in Graz, Styria (Camp et al. 2019). In comparison to other spreading aedine mosquito species like *Ae. albopictus*, *Ae. japonicus* remains insufficiently known outside its native range. (Juliano & Lounibos 2005): vector competences and host preferences in the field were not fully examined so far (Andreadis et al. 2001).

In this study, we monitored the range expansion of *Ae. japonicus* in the province Burgenland, Austria, and provide data detailing range expansion speed over six years of sampling, from 2014–2019.

Methods

Mosquitoes were sampled on a regularly basis as part of a mosquito monitoring programme carried out from 2014 to 2019. The 13 sampling sites were distributed across the province of Burgenland (Austria), located in the districts of Güssing, Jennersdorf, Oberwart, Oberpullendorf, Mattersburg and Neusiedl am See. The trapping site in Neusiedl am See,

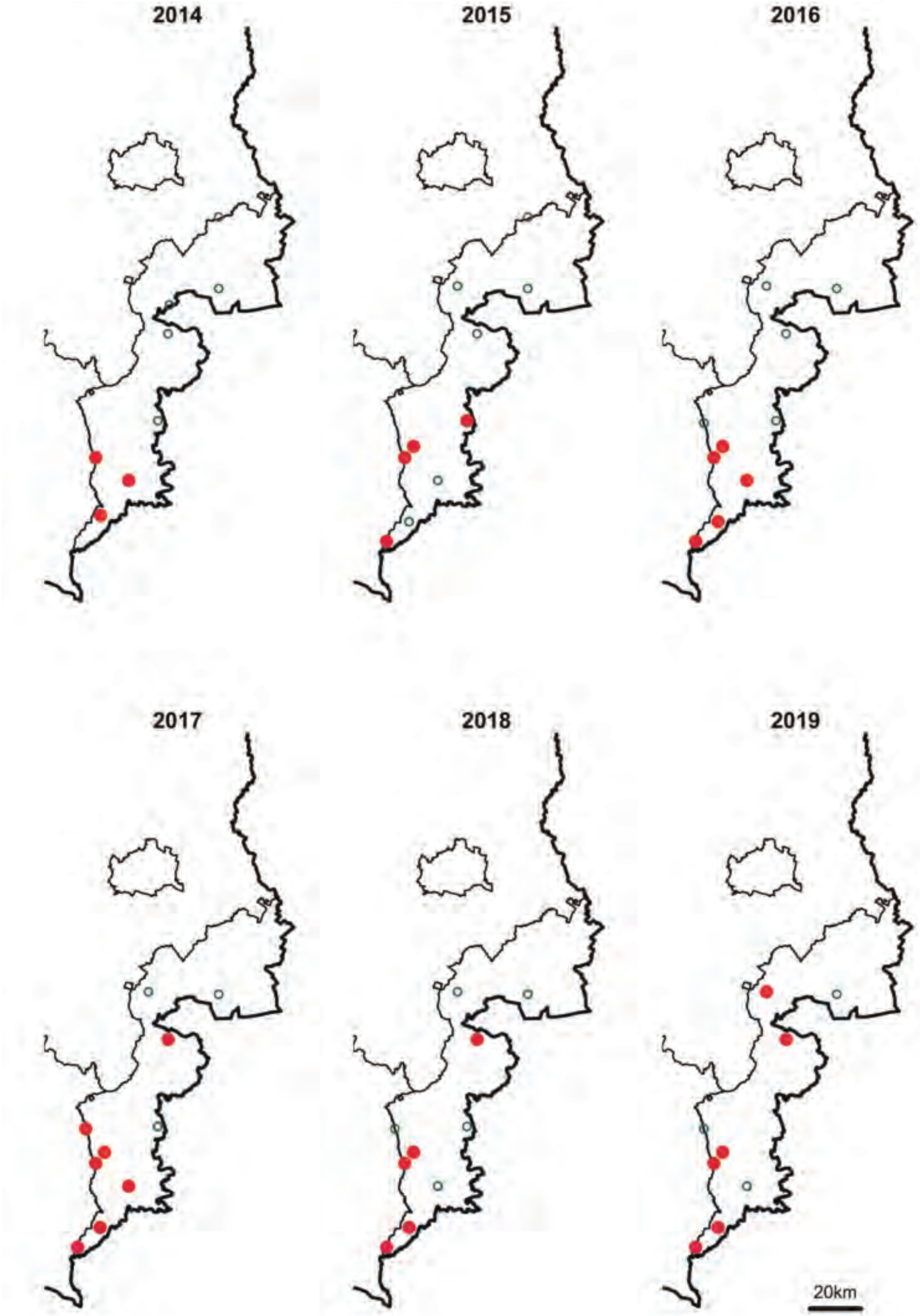

Fig. 2: Sampling sites positive for *Ae. japonicus* shown as red dots, negative sampling sites as green circles. Note that the number of sampling sites varies. Data source province borders: NUTS units, Statistik Austria - data.statistik.gv.at

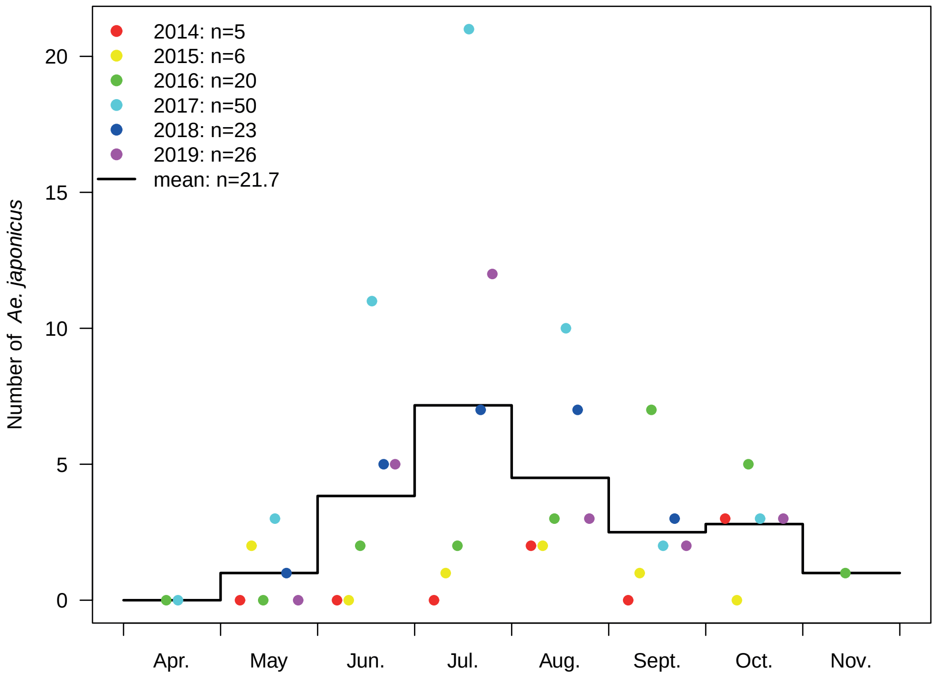

Fig. 3: Seasonal distribution of the captured *Ae. japonicus* females. n gives the total number of *Ae. japonicus* in each of the study years and the mean number per year.

at the biological station in Illmitz, is part of LTER (Long-Term Ecosystem Research) Austria. All districts were monitored continuously throughout the entire sampling period of six years. The amount of trapping sites, however, changed between the years with their accessibility. Female mosquitoes were sampled from April to October/ mid-November biweekly for a 24-hour time period using Biogents® Sentinel traps (Regensburg, Germany) baited with carbon dioxide as attractant. Afterwards mosquitoes were frozen at a temperature of -20° until identification to species- or species-complex-level using morphological characteristics using the keys of of Becker et al. (2010) and Gunay et al. (2018). Then, individuals partly missing identification characteristics for a reliable species determination, were further analysed using molecular tools as internal quality control. For this purpose, genomic DNA was extracted from 3 legs or the head capsule of single specimens using the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. Partial amplification of approximately 700 bp fragments of mitochondrial cytochrome c oxidase subunit I (COI) was performed using primers LCO1490 and HCO2198 as described in Folmer et al. (1994).

Results

Throughout the sampling period from 2014–2019, 7542 female mosquitoes were captured, mostly *Culex pipiens/Cx. torrentium* (45.2 %), *Cx. modestus* (20.6 %) and *Coquillettidia richiardii* (8.6 %). A total of 130 *Ae. japonicus* (1.7 %) were collected.

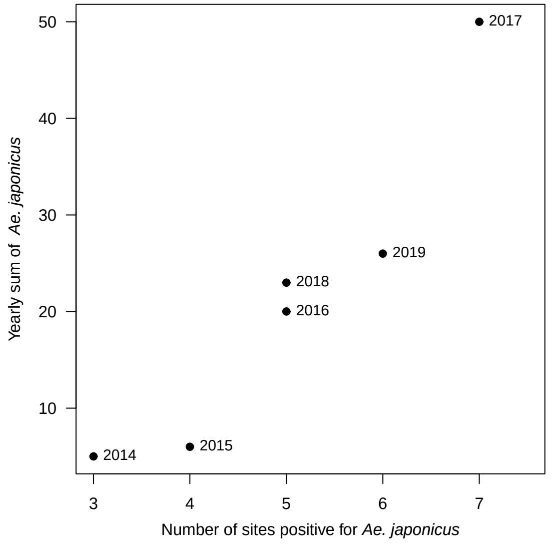

Fig. 4: Yearly total sum of *Ae. japonicus* and the total number of sites found with this species in each year.

The first individuals of this species were detected in 2014, at the three southernmost sampling sites, located in the districts Güssing and Jennersdorf (Fig. 2). In the following years, *Ae. japonicus* was detected at more northern sampling sites. The mean northward dispersal of this species was 13.6 km/year (sd=20.87). In 2019, its northernmost distribution was in the district Mattersburg, which is located 67.9 km north of the sampling site in Güssing (which was the northernmost distribution in 2014).

The activity period of *Ae. japonicus* ranged from May to November. In July, the highest numbers (mean=7.2, sd=8.13) of this species were recorded, followed by August (mean=4.5, sd=3.27) and June (mean=3.8, sd=4.17) (Fig. 3).

The total number of *Ae. japonicus* increased with the number of sampling sites at which the species could be recorded (r= 0.9467; Pearsons correlation test: p= 0.004, t = 5.8938, df = 4; Fig. 4). The number of positive sites as well as the number of captured *Ae. japonicus* generally increased with the years. In 2017 an exceptionally high number of *Ae. japonicus* specimens was found, followed by a yet unexplained decrease in the following two years.

Discussion

Based on our long-term mosquito monitoring data in Burgenland we could demonstrate northwards range expansion of the Asian bush mosquito in Burgenland. Due to the restrictions of the monitoring program, we could only detect an expansion northwards, but we expect similar dispersal in neighbouring regions (Styria; Hungary). Although we focused only on a small part of the distribution area of this species, dominated by pannonic climate, the results of this study could help to predict future range expansion in *Ae. japonicus*, if effects of climatic conditions and land cover can be accounted for (Kerkow et al. 2019).

The first record of *Ae. japonicus* in Austria in 2011, in the region of Kreuzberg in Styria (Seidel et al. 2012), was followed by a rapid spread of this species in all directions in the following years (Schoener et al. 2019, Seidel et al. 2016). The first specimens of *Ae. japonicus* in Burgenland were collected in 2012, in the very south of this province (Seidel et al. 2016). Our study thus indicates a continuous increase in the range of this species, most probably driven by natural factors like active dispersal or wind.

In Austria, *Ae. japonicus* has also been detected in Tyrol, in 2015. However, it is unlikely that this population in western Austria is connected with the one in south-eastern Austria and seems to be the result of a distinct introducing event (Koban et al. 2019). As in *Ae. albopictus*, the most important mode of long-distance dispersal of *Ae. japonicus* within Europe appears to be passive transportation by vehicles, which makes repeated introduction events likely (Werner et al. 2012, Becker et al. 2013, Kampen & Werner 2014, Eritja et al. 2017).

We found *Ae. japonicus* on the wing from May to November, with peak abundances in July. This concurs with other findings from Austria, were eggs of this species could be detected in ovitraps from July to September (Schoener et al. 2019, Fuehrer et al. 2020). Previous studies, however, often report a different pattern, where adult *Ae. japonicus* are active earlier in the spring and later in the fall compared to ecologically similar species (Kaufman & Fonseca 2014). Dussault et al. (2018) could show that *Ae. japonicus* peaks in September in the Greater Golden Horseshoe region in Canada, when the abundance of the other mosquitoes sharing an ecological niche with *Ae. japonicus* decreases. As a different set of mosquito species shares their larval habitat with *Ae. japonicus* in Austria it could be possible that they can adapt their phenology to co-occurrence patterns of other species. However, a wide range of other factors including climatic and meteorological parameters is likely to affect phenological patterns and this phenomenon needs to be investigated further.

In our study, *Ae. japonicus* represented only a fraction of the total catch, although we focussed on the preferred landcover type (suburban/rural areas) (Chaves et al. 2020). As this species is a new element in the Austrian fauna, it may not yet have established stable populations in equilibrium with the environment. Still, *Ae. japonicus* is common but does not occur in high abundances in its native range, Japan and Korea. This pattern is also observed in areas were the species was introduced and could establish (Kaufman & Fonseca 2014, Dussault et al. 2018, Wagner et al. 2018). It is therefore also possible that *Ae. japonicus* is simply not attracted to BG-Sentinal traps (similar to other mosquito species), and that population sizes cannot be estimated based on sampling efforts using this trap type.

Larvae of *Ae. japonicus* often share their habitat with larvae of other mosquito species, but it remains unclear whether there is any significant interaction between them, both in terms of resource competition or potential displacement. Therefore, potential invasiveness of this species with respect to other mosquitoes has to be critically appraised. Mosquito species sharing their larval habitat with *Ae. japonicus* in Austria and thus potentially affected by this species are *Ae. geniculatus*, *Anopheles plumbeus*, *Culiseta longiareolata*, members of the *Culex pipiens* complex as well as *Cx. torrentium* and probably also members of the *An. maculipennis* complex that either oviposit in water-filled tree holes or small artificial containers (Zittra & Waringer 2015, Zittra et al. 2015). While *Ae. japonicus* was reported to displace native mosquito species (i.e. *Ae. triseriatus* and *Ae. atropalpus*) in other countries, there is no clear evidence for direct larval competition with the northern house mosquito, *Cx. pipiens*, or other mosquito species native to Austria (Kaufman & Fonseca 2014). Also, effects on other biota remain to be investigated.

At present, Austrian populations of *Ae. japonicus* cannot be considered as invasive as there is no evidence that this mosquito is able to outcompete or affect autochthonous species. However, detailed long-term investigations on *Ae. japonicus* habitats (both larval and adult), associated species and their interactions are needed to characterize invasiveness of this species in Austria. Yet, if abundances of *Ae. japonicus* remain at a low level in Austria, its influence on the ecosystems and its relevance as a disease vector may remain limited.

Zusammenfassung

Die potenziell invasive Stechmückenart *Ae. japonicus* (Japanische Buschmücke) wurde im Jahr 2000 nach Europa eingeschleppt und 2011 erstmals in Österreich nachgewiesen. Im Rahmen eines Stechmücken-Monitoring-Programms (2014–2019) wurde die Ausbreitung von *Ae. japonicus* im Bundesland Burgenland (Österreich) untersucht. Während des gesamten Beobachtungszeitraums wurden 130 *Ae. japonicus* Weibchen gefangen. Die ersten Exemplare wurden 2014 an den südlichsten Fallenstandorten gefangen. In den folgenden Jahren verbreitete sich diese Art nach Norden, im Durchschnitt mit 13,6 km/Jahr (sd = 20,87). Der nördlichste Punkt des Verbreitungsgebiets befand sich 2019 im Bezirk Mattersburg, 67,9 km nördlich des nördlichsten Verbreitungsareals von 2014 (Güssing). Adulte *Ae. japonicus* wurden von Mai bis November gefangen, wobei die höchste Anzahl in Juli und August gefangen wurde. Die kontinuierliche Ausbreitung dieser Art von Süden nach Norden legt nahe, dass diese durch natürliche Faktoren, wie aktive Verbreitung oder Windverfrachtung, bedingt ist. Die Ergebnisse dieser Studie zeigen, dass *Ae. japonicus* im Burgenland weit verbreitet aber nicht sehr häufig ist. Derzeit gibt es keine Hinweise, dass *Ae. japonicus* in Österreich invasives Verhalten zeigt. Weitere Untersuchungen der österreichischen Population von *Ae. japonicus* sind notwendig, um ihre Wechselwirkungen mit einheimischen Arten zu untersuchen und einen Status der Invasivität zu definieren.

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