Monitoring of alien mosquitoes in Western Austria (Tyrol, Austria, 2018)

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Abstract

Mosquitoes are of major importance to human and animal health due to their ability to transmit various pathogens. In Europe the role of mosquitoes in public health has increased with the introduction of alien Aedes mosquitoes such as the Asian tiger mosquito, Aedes albopictus; the Asian bush mosquito, Ae. japonicus; and Ae. koreicus. In Austria, Ae. japonicus has established populations in various regions of the country. Aedes albopictus is not known to overwinter in Austria, although isolated findings of eggs and adult female mosquitoes have been previously reported, especially in Tyrol. Aedes koreicus had not so far been found in Austria. Within the framework of an alien mosquito surveillance program in the Austrian province of Tyrol, ovitraps were set up weekly from May to October, 2018, at 67 sites—17 in East Tyrol and 50 in North Tyrol. Sampling was performed at highways and at urban and rural areas. DNA obtained from mosquito eggs was barcoded using molecular techniques and sequences were analysed to species level. Eggs of alien Aedes species were found at 18 out of 67 sites (27%). Both Ae. albopictus and Ae. japonicus were documented at highways and urban areas in both East and North Tyrol. Aedes koreicus was found in East Tyrol. During this mosquito surveillance program, eggs of Ae. albopictus, Ae. japonicus, and Ae. koreicus were documented in the Austrian province of Tyrol. These findings not only show highways to be points of entry, but also point to possible establishment processes of Ae. japonicus in Tyrol. Moreover, Ae. koreicus was documented in Austria for the first time.

Author summary

The importance of mosquitoes for Public Health in Europe increased dramatically with the introduction of alien species considered to be competent vectors of important human pathogens (e.g. dengue, chikungunya, and Zika viruses), which autochthonous mosquitoes are not yet known to transmit. The Asian tiger mosquito (Aedes albopictus), the Asian bush mosquito (Aedes japonicus), and Aedes koreicus are particularly of relevance, as they are expanding their range in Europe. Tyrol, a region in the Alps with main transport routes from Italy to Germany is of high relevance for the spread of potential invasive,
Introduction

Blood-feeding mosquitoes play a major role in the transmission of pathogens. In Europe, their importance has increased in the past decade—mainly because of the introduction and establishment of invasive mosquitoes able to transmit pathogens which autochthonous mosquitoes are not able (or not known to be able) to transmit (e.g. chikungunya, dengue, and Zika viruses) [1]. Especially the Asian tiger mosquito, *Aedes* (*Stegomyia*) *albopictus* (Skuse, 1894), Asian bush mosquito, *Aedes* (*Hulecoeteomyia*) *japonicus* (Theobald, 1901), and *Aedes* (*Hulecoeteomyia*) *koreicus* (Edwards, 1917) have expanded their range in Europe in the past decade [2, 3]. A recent survey on alien species and human health in Austria lists invasive vectors such as *Ae. albopictus* as those alien species posing the most severe challenges [4].

The Asian tiger mosquito (*Ae. albopictus*) originates from subtropical and tropical Asian-Pacific regions and can actually be found on all continents with the exception of Antarctica [5]. The first report of Asian tiger mosquitoes in Europe originates from Albania in 1979 [6], but its European spread started from Italy (first discovery in 1990 in Genoa or 1991 in Padova). Today, *Ae. albopictus* has been reported from more than 25 countries in Europe and established populations are known in at least 19 European countries [3]. Although overwintering and established populations are not known from Austria, this species has been reported to be introduced from Germany [7, 8], South Tyrol (northern Italy) [9], and other neighbouring regions. *Aedes albopictus* is of major public health concern, being a vector not only of various arboviruses such as dengue, chikungunya, Zika, and West Nile [5, 10], but also filarioid helminths [11]. Local outbreaks of chikungunya and dengue have been documented in Europe—two viruses not known to be transmitted by mosquitoes’ native in Europe (e.g. [12, 13]).

The native distribution area of the Asian bush mosquito, *Aedes japonicus*, is limited to temperate climatic regions of East Asia (China, Korea, Japan and south-eastern Russia). It is known as one of the most invasive mosquito species worldwide and has colonised 15 countries in Europe (e.g. Switzerland, Germany, Austria, France, Slovenia; summarized in [14, 15]) since its introduction. Under laboratory conditions *Ae. japonicus* is a competent vector of various pathogens: West Nile virus, Japanese encephalitis virus, chikungunya virus, dengue virus, *Dirofilaria repens*, and *D. immitis* (summarized in [14, 16, 17]). In field-sampled mosquitoes the Japanese encephalitis virus (in its native distribution range), but also West Nile Virus, La Crosse virus, and Cache Valley Virus (in the USA) have been detected (summarized in [14]). Recently the Usutu virus was documented in *Ae. japonicus* caught in the field in Graz, Austria [18].

*Aedes koreicus* is naturally distributed in East Asia from Japan, Korea, China, to parts of Eastern Russia. This invasive species was first documented in Europe in 2008 in a small area of 6 km² in Belgium [19, 20]. In 2011, *Ae. koreicus* was found in the Veneto Region, North-Eastern Italy [21], where it spread rapidly [22–25]. Recently it was documented in the Northwest of Italy (Liguria: [26]). After the first findings in Italy, *Ae. koreicus* was also documented along the Swiss-Italian border, in Germany, Slovenia, and Hungary [27–32]. Under laboratory conditions, this mosquito is a competent vector for chikungunya virus and *Dirofilaria immitis* [33,
In the current study, alien mosquito species were monitored in the Western Austrian province of Tyrol. The study was conducted within the framework of the alien mosquito monitoring program of the federal state of Tyrol to evaluate the presence of alien Aedes spp. using ovitrapping at highways, but also in populated areas in Tyrol.

Materials and methods

Study area and sampling

Tyrol is a federal state in Western Austria and comprises the northern and eastern part of the historical princely county of Tyrol. North Tyrol borders on Germany (Bavaria) in the North, Vorarlberg in the West, Salzburg in the East, and Italy (South Tyrol) and Switzerland (Graubünden) in the South. East Tyrol shares its border with the Province of Belluno, Veneto region (Italy) and South Tyrol in the West. Potential larval sites (e.g. parking lots and petrol stations at highways, but also urbanized areas) were chosen as sampling sites. Ovitraps were set up weekly from May to October 2018 (calendar week 18–40) at 67 sites– 17 in East Tyrol and 50 in North Tyrol. They were installed in cities and villages (n = 53), at the Inn Valley Highway between Innsbruck and Kufstein (A12; n = 7), and the Brenner Highway between the Italian border and Innsbruck (A13; n = 7).

Ovitraps are widely used for surveillance of alien and invasive Aedes species as described elsewhere (e.g. [36]), and the same technique was used for the monitoring in 2017 [37]. Two conical black 500-ml cups filled with approximately 400 ml of water were set up per site. Wooden paddles were inserted as substrate for mosquito oviposition. Paddles were collected weekly and analysed for the presence of mosquito and other insect eggs under a dissection microscope. From each paddle Aedes eggs were pooled and transferred to 1.5-ml Eppendorf tubes for molecular analysis. Samples were stored at -20°C until further molecular analysis.

Molecular mosquito specification

After homogenisation of eggs in a TissueLyser II (Qiagen, Germany) with two ceramic beads (2.8 mm Precellys Ceramic Beads, VWR, Germany) as described previously [37], DNA was isolated using the Qiagen DNeasy Blood&Tissue kit (Qiagen, Germany) according to the manufacturer’s instructions. To identify insect species, barcoding was performed within the mitochondrial cytochrome oxidase subunit I (mt COI) gene using the primers LepF1 and LepR1 [38]. PCR products were sequenced at LGC Genomics GmbH, Germany. Resulting sequences were compared to sequences available on BOLD Systems and GenBank databases. Aligned sequences were uploaded to GenBank (MN103383- MN103400).

Results

Eggs of alien Aedes species were found at 18 of 67 Tyrolean sites (27%). (Potentially) invasive mosquito species were more common at highways (7/14; 50%) than in other areas (11/53; 21%; Table 1). At the Inn Valley Highway A12 (5/7; 71%) more sites were positive for mosquito eggs than at the Brenner Highway A13 (2/7; 29%). In East Tyrol at 41% of the sites (7/17) eggs of alien mosquitoes were documented, whereas in North Tyrol 8% (4/50; only in urban areas–Innsbruck and Kufstein) gave positive findings (Table 2). Given the relatively small sample sizes, these differences were not statistically significant.

Aedes albopictus was documented at six of 67 sites (9%; Fig 1). In total, three out of 14 sites at highways were positive (21%). However, in this study tiger mosquitoes were only found at
the Inn Valley Highway (3/7; 43%). No *Ae. albopictus* eggs were detected at the Brenner Highway. However, eggs were detected in urban areas in East Tyrol (1/17; 6%) in Lienz and in North Tyrol (2/36; 6%) in Innsbruck and Kufstein. Sequence analysis revealed two haplotypes (100% identity to MH817558 and KC690954).

*Aedes japonicus* was documented at 15 of 67 sites (22%; Fig 1) both at highways (6/14; 43%) and other areas (9/53; 17%). This species was detected at the Brenner Highway (2/7; 29%) close to the Italian border to South Tyrol, and the Inn Valley Highway (4/7; 57%). In East Tyrol, it was found in rural and urban areas (6/17; 35%). In North Tyrol (excluding highways) eggs of *Ae. japonicus* were detected at three of 50 sampling sites (6%) all located in Kufstein. Genetic analysis revealed 10 haplotypes of *Ae. japonicus* within the mt COI barcode region (see Tables 1 and 2).

*Aedes koreicus* eggs were found at a single site in Lienz, East Tyrol, in August 2018 (Fig 1). At sequence analysis, the sample was 100% identical to *Ae. koreicus* KM258298 collected in Maasmechelen, Belgium [39].

Additionally, eggs of autochthonous mosquito species were documented in this study, namely *Aedes geniculatus* (July and September 2018), *Aedes* sp. (September 2018; MN103396), *Anopheles plumbeus* (October 2018), *Culex pipiens* complex (July 2018), and *Culex torrentium* (June and August 2018; MN103395). The following dipteran non-mosquito insect eggs were documented: 0.4–0.5 mm long brownish eggs of *Clogmia albipunctata* (June 2018; Psychodidae; MN103400), 0.6 mm brownish eggs of *Sphegina clunipes* (July 2018; Syrphidae; MN103398), and 0.3 mm long white eggs of *Chloropidae* (August 2018; grass flies; MN103397). At sequence analysis of pooled samples, double-peaks indicating numerous different *Aedes* species were observed at one sample at Kufstein-Airport (September 18th 2018). However, it might have failed at other pooled samples.

**Discussion**

The first report of *Ae. albopictus* in Austria was based on findings of immature stages and a single female mosquito in 2012 in Jennersdorf, Burgenland, and *Ae. albopictus* larvae in Angath, Tyrol, approximately 2 km from the Inn Valley Highway [40]. Reports of single female mosquitoes and eggs were subsequently documented along the Inn Valley Highway, which
prompted a continuous monitoring of this area using ovitraps. Our findings of *Ae. albopictus* eggs along the Inn Valley Highway were expected. In 2017, eggs of tiger mosquitoes were documented in two of the five positive sites from 2018 (Rest stop Weer South and Parking Site Müns ter South; [37]). In Bavaria (e.g. Kiefersfelden close to the Austrian border and Kufstein), *Ae. albopictus* was also found at service stations, associated with transit road traffic [41]. The dispersal of adult *Ae. albopictus* by car has recently been shown in Spain [42].

| Location | Longitude | Latitude | Date | Species          | Number of *Aedes* spp. eggs present | Corine Land Cover (Level 3) | GenBank ID |
|----------|-----------|----------|------|------------------|------------------------------------|-----------------------------|------------|
| East Tyrol |           |          |      |                  |                                    |                             |            |
| Lienz—cemetery St. Andrä/8 | 46.834270 | 12.759662 | July 11 | *Ae. japonicus* | 13 | Discontinuous urban fabric | MN103384 |
| Lienz—red cross/9 | 46.834190 | 12.766865 | July 4 | *Ae. japonicus* | 62 | Discontinuous urban fabric | MN103384 |
|           |           |          | July 25 | *Ae. japonicus* | 48 |                          | MN103386 |
|           |           |          | Aug. 9 | *Ae. japonicus* | 141 |                                    | nd         |
|           |           |          | Aug. 16 | *Ae. koreicus* | 22 |                                | MN103399 |
| Lienz—Fischwirbbrücke/10 | 46.831458 | 12.769565 | July 25 | *Ae. japonicus* | 20 | Continuous urban fabric | MN103387 |
| Lienz—Tiroler Strasse/11 | 46.827560 | 12.767468 | July 18 | *Ae. japonicus* | 51 | Continuous urban fabric | MN103384 |
| Lienz—near fire brigade/12 | 46.826782 | 12.759982 | July 11 | *Ae. albopictus* | 12 | Discontinuous urban fabric | MN103394 |
|           |           |          | July 18 | *Ae. albopictus* | 137 |                                      | nd         |
| Tristach/13 | 46.814563 | 12.805695 | Sep. 20 | *Ae. japonicus* | 54 | Non-irrigated arable land | MN103389 |
| Nörsacher Teiche/14 | 46.766280 | 12.931955 | Sep. 27 | *Ae. japonicus* | 187 | Land principally occupied by agriculture, with significant areas of natural vegetation | nd |
| North Tyrol |           |          |      |                  |                                    |                             |            |
| Innsbruck—Badhaus/15 | 47.281683 | 11.406097 | Aug. 8 | *Ae. albopictus* | 3 | Discontinuous urban fabric | MN103394 |
| Kufstein—Site 1/16 | 47.556250 | 12.118567 | July 26 | *Ae. japonicus* | 13 | Industrial or commercial units | MN103388 |
|           |           |          | Aug. 8 | *Ae. japonicus* | 86 |                                      | nd         |
|           |           |          | Aug. 29 | *Ae. japonicus* | 34 |                                | MN103391 |
| Kufstein—Airport/17 | 47.566167 | 12.126500 | June 14 | *Ae. albopictus* | 11 | Discontinuous urban fabric | MN103394 |
|           |           |          | July 12 | *Ae. japonicus* | 7 |                                      | nd         |
|           |           |          | Aug. 29 | *Ae. japonicus* | 57 |                                | MN103391 |
|           |           |          | Sep. 26 | *Ae. japonicus* | 66 |                                | MN103392 |
| Kufstein—Site 2/18 | 47.565967 | 12.145733 | June 21 | *Ae. japonicus* | 119 | Discontinuous urban fabric | MN103383 |
|           |           |          | Sep. 5 | *Ae. japonicus* | 151 |                                | MN103385 |

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In 2017, in East Tyrol, a single *Ae. albopictus* egg was reported from an ovitrap set up in Tassenbach. This sampling site was negative for tiger mosquitoes in 2018, but *Ae. albopictus* was found in Lienz on July 11th and 18th 2018. In North Tyrol, *Ae. albopictus* was found in Kufstein in 2017 [37] and in Innsbruck and Kufstein in 2018. These findings indicate not only introduction but also a possible establishing process, but further studies are needed to prove this hypothesis. Overwintering populations and possible establishment of tiger mosquitoes has been reported from neighbouring Italy (e.g. South Tyrol and Trento; e.g. [43]) and Germany (e.g. Freiburg; [44, 45]) but so far not from Tyrol or other parts of Austria. The only Austrian province (almost) free (exception Kufstein area) of these mosquito species was Tyrol with the "Swiss/Vorarlberg" *Ae. japonicus* population in the West, the "Carinthian/Italian" population in the South and the "Salzburg/Upper Bavarian" population in the East. In 2017, no *Ae. japonicus* eggs were reported [37]. By contrast, 15 of 67 sampling sites were positive for *Ae. japonicus* eggs in 2018. *Aedes japonicus* was documented at two petrol stations at the Brenner Highway close to the Italian border and at the Inn Valley Highway, indicating vehicular traffic as a route of introduction. However, this mosquito species might also have reached this area by active flight. Moreover, *Ae. japonicus* was found in East Tyrol (e.g.
Lienz), indicating dispersal to this area from Italy in the South or Carinthia in the East. The findings of *Ae. japonicus* in Kufstein were associated with populations in Upper Bavaria/Germany. According to Koban et al. [51], four populations of Asian bush mosquitoes are currently present in Central Europe. This study indicates that the two biggest populations (Western-German/Swiss/French and Southern-Austrian/Slovenian/Italian) might encounter each other in North Tyrol. Population genetic studies (nad4 mitochondrial locus and microsatellite analysis [52]) might resolve this question, but a recent study demonstrated that the origin of entry into Germany cannot be clarified ten years after the first detection in that country [14]. Active spread, re-introduction, and carry-overs may take place regularly [14].

Although *Ae. koreicus* was reported in North-East Italy and Slovenia, this mosquito species had not been reported from Austria until now. In the present study, *Ae. koreicus* eggs were found for the first time in August 2018 in Lienz, East Tyrol. The Italian region Veneto (where *Ae. koreicus* is known to be present) borders onto East Tyrol [48]. However, with the findings of eggs in ovitraps at only one location, it remains unclear if this mosquito has spread to Austria or if it was an isolated introduction. Establishment in hilly and pre-alpine areas up to an altitude of 800 m in Italy [21, 22] indicates that establishment and further distribution can be expected in Austria in the coming years.

Fifty culicid species (Diptera: Culicidae; genera: *Aedes*, *Anopheles*, *Culex*, *Coquillettidia*, *Culiseta*, *Ochlerotatus*, *Orthopodomyia*, and *Uranotaenia*) have been detected in Austria so far [53]. With the finding of *Ae. koreicus* this number increases to 51 species.

This study has some limitations. Barcode primers (mt COI) known to bind these mosquito species were used. Analysis of pooled mosquito eggs might have meant that mosquito species were overlooked if more than one mosquito species laid eggs on a paddle, but this technique had been chosen because until this monitoring program only *Ae. albopictus* was reported in the studied area (37).

We focused on alien *Aedes* mosquitoes and therefore used ovitraps only. This technique is cheap, easy to use, and an effective tool for monitoring alien mosquito species. Further research (e.g. inclusion of adult mosquito sampling) and surveillance is needed to evaluate overwintering, establishment, and invasive behaviour of *Ae. albopictus*, *Ae. japonicus*, and *Ae. koreicus* in the Austrian province of Tyrol, but also in other regions in Austria.

Using ovitraps, *Ae. albopictus*, *Ae. japonicus*, and *Ae. koreicus* were found in the Austrian province of Tyrol. *Aedes albopictus* and *Ae. japonicus* were both documented not only at highways but also in urban areas in Tyrol. These findings not only demonstrate that highways are points of entry but also point to possible establishment processes in Tyrol. To the best of our knowledge this is the first report of the presence of *Ae. koreicus* in Austria. The risk of the establishment of especially *Ae. albopictus* (but also *Ae. koreicus* and *Ae. japonicus*) in North and East Tyrol is clear, and informing the public and stakeholders about measures to hamper this development is highly recommended.

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