

# First evidence and distribution of the invasive alien mosquito *Aedes japonicus* (Theobald, 1901) in Luxembourg

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**Abstract.** The invasive mosquito species *Aedes japonicus* (Theobald, 1901) was first recorded in Luxembourg on 4 July 2018 in Stolzembourg in the middle Our valley on the German border. During three consecutive field missions in 2018, 106 sites were inspected, including 91 in Luxembourg, 12 in Germany, 2 in Belgium and 1 in France. *Aedes japonicus* was detected in 16 sites, the colonised area being estimated to cover at least 550 km<sup>2</sup>, located in the East of the Grand Duchy, from the middle Our valley in the North to Ernster in the South, and as far as Kautenbach and Ettelbruck in the West. Considering a colonising speed of 10–20 km / year, the species might colonise the whole territory of the Grand Duchy by the end of 2020. An environmental risk assessment according to the ISEIA protocol revealed that the species shows a high dispersal potential, a medium risk of colonisation of high conservation value habitats, a medium risk of adverse impacts on native species and a low risk of alteration of ecosystem functions. A detailed risk assessment according to the Harmonia+ protocol confirms the relatively low environmental impact of *Ae. japonicus* and is being compared to the risk assessment of *Ae. albopictus*. Eight other mosquito species were observed in Luxembourg during these missions: *Aedes geniculatus* (Olivier, 1791), *Anopheles claviger* s.s. (Meigen, 1804), *An. plumbeus* (Stephens, 1828), *Culex hortensis* Ficalbi, 1889, *Cx. pipiens* Linnaeus, 1758, *Cx. torrentium* Martini, 1925, *Culiseta annulata* (Schrank, 1776), *Cs. longiareolata* (Macquart, 1838). Both *Cx. hortensis* and *Cs. longiareolata* are new to the fauna of Luxembourg.

**Keywords.** *Aedes japonicus*, *Culex hortensis*, *Culiseta longiareolata*, Culicidae, invertebrates, invasive alien species, risk assessment, ISEIA, Harmonia+, Luxembourg.

## 1. Introduction

*Aedes* (*Hulecoeteomyia*) *japonicus* (Theobald, 1901), the East Asian bush mosquito (Fig. 1), is a species (Diptera: Culicidae) originally native to East Asia (southern China, Japan, Korea, southern Russia, and Taiwan). Its most common synonyms are *Hulecoeteomyia japonica* and *Ochlerotatus japonicus* but we here follow the revised Aedini nomenclature by Wilkerson et al. (2015) and the Systematic Catalog of Culicidae (Gaffigan et al. 2015).

The *Ae. japonicus* taxon comprises 4 subspecies with specific geographical distributions, namely *Ae. japonicus amamiensis* (Ryu Kyu Amami Guntô Island), *Ae. japonicus japonicus* (Palearctic Japan, Korea, Russian Primorsky Krai), *Ae. japonicus yaeyamen-*

*sis* (Ryu Kyu Yaeyama Guntô island), and *Aedes japonicus shintienensis* (Taiwan and probably southern China) (Schaffner et al. 2003, Kampen & Werner 2014). Their morphology differs mainly in tibia ornamentation (Tanaka et al. 1979). To date, only *Ae. japonicus japonicus*, hereafter given as *Ae. japonicus*, has been shown to spread outside its native range.

Immatures of *Ae. japonicus* breed in a variety of natural and artificial water-holding containers, such as tree holes, rock holes, tyres, barrels, buckets, drinking troughs, tarpaulins or road drains (Medlock et al. 2015). These breeding sites are often rich in organic matter, containing decaying leaves (Kampen & Werner 2014). The species shows pref-



Fig. 1. Biting female of the East Asian bush mosquito *Aedes japonicus*. Typical white stripes on black background are visible on legs and abdomen, but dorsal part of thorax (scutum) is brownish, contrary to *Ae. albopictus* which shows a medio-dorsal white line on black background. Photo credit: Francis Schaffner.

erence to shaded places for larval habitats but also adults more frequently bite people under trees and bushes than in open land (Schaffner F., personal observations). Eggs are frost and desiccation resistant, and therefore can be transported over long distances when laid in e.g. used tyres.

Even though the species is not considered an important vector of pathogens in its native range, there is a concern about its possible threats to public health as well as to animal health as a potential vector of West Nile virus and other various types of encephalitis viruses (Schaffner et al. 2013b, Kampen & Werner 2014).

### Worldwide spread and distribution of *Aedes japonicus*

Being native to East Asia, *Ae. japonicus* was detected out of its original distribution range for the first time in the early 1990s in New Zealand where it did not establish, though. In 1998, established populations were reported from the eastern USA, most likely as a result of introductions several

years earlier. After a massive spread the mosquito is now widely distributed in eastern North America including Canada and two US states on the western coast (Kampen & Werner 2014).

In the year 2000, *Ae. japonicus* was demonstrated for the first time in Europe, in Normandy (Orne) in northern France, from where it could be eliminated (Schaffner et al. 2003, 2009). It was then reported in 2002 from Belgium at a tyre depot and presence as adults and larvae was confirmed in 2007 and 2008 (Versteirt et al. 2009). Like in France, the mosquito species was most likely introduced through the trade of used tyres. In Belgium, its population was well established at the tyre company site and its surroundings but did not appear to be spreading. From 2012 onwards the population was controlled and considered eliminated in 2015 (Versteirt et al. 2017).

*Aedes japonicus* was detected in Switzerland in 2008 following reports of a biting nuisance and subsequent surveys revealed a 1.400 km<sup>2</sup> colonised zone in the country but also crossing the border into Germany. This was the first detection of invasive mosquito spreading in central Europe (Schaffner et al. 2009). Subsequent studies in Germany showed a wide distribution and several populations of the mosquito in various federal states (Kampen et al. 2012, Werner & Kampen 2013).

In 2011, the species was found in south-eastern Austria (Styria) and neighbouring Slovenia. In 2013, a population was detected in the central part of the Netherlands, specimens were collected in southern Alsace, France, and the complete north-eastern part of Slovenia was found colonised, with specimens also present across borders in adjacent Croatia. Apparently, at the end of 2013 a total of six populations occurred in Europe although it is not clear whether all of them are completely isolated (Kampen & Werner 2014). Similarly, it is not known whether these populations go back to the same number of introductions. While entry ports and long-distance continental migration routes are obscure, it is likely that the international used tyre trade is the most

important mode of intercontinental transportation of the mosquito.

Since this overview by Kampen and Werner (2014) the Asian bush mosquito has spread further throughout parts of Europe including Liechtenstein and Hungary (Seidel et al. 2015). As for the surroundings of Luxembourg and at the time of the survey (2018), the species was still considered eliminated from the Province of Namur (Belgium), but established in Lelystad (Netherlands), in North Rhine-Westphalia and Rhineland-Palatinate (Germany), and in Bas-Rhin, Haut-Rhin, and Vosges (France) (ECDC 2018). Very recently it was reported as introduced and established in Spain (Eritja et al. 2019), and further spreading in Germany (Koban et al. 2019).

### Context and aim of the study

On 4 July 2018, a private individual captured three biting mosquitoes with an unusual appearance in and around his home on Rue du Faubourg in Stolzembourg (municipality of Putscheid), in the Our valley at the north-east border to Germany. These specimens were identified as belonging to the invasive alien species *Ae. japonicus* (Theobald, 1901) by the Luxembourg National Museum of Natural History (MNHN). This mosquito, besides being exotic and invasive, is also known to generate nuisance in some context and has the potential to contribute to pathogen transmission to both humans and animals (i.e. West Nile virus). Therefore the National Health Directorate (Ministry of Health, Luxembourg) entrusted several short field missions in order, first, to confirm the species' presence and establishment, and later, to assess its spread and distribution over the Grand Duchy. This paper describes the results of a two-step investigation into the distribution of this species in Luxembourg and surrounding regions. To assess the risk that this invasive mosquito species represents to biodiversity and its impacts on several targets, we performed a standard risk assessment applying two protocols developed by the Belgian Forum on Invasive Species (BFIS) and widely used in north-western Europe: ISEIA and Harmonia+.

## 2. Methods

### 2.1. Field surveys

In a first step, localities to be prospected were selected on map and satellite image according to their distance from the original known colonised site (Stolzembourg) and their ecological potential (e.g. proximity to afforestation, suspicion of presence of favourable sites) or local knowledge of the terrain. In a second step the sites were visually identified on location, according to the presence of potential larval breeding sites, such as water containers, rainwater barrels, tyres, tarpaulins, manholes, water troughs, fountains, vases (Fig. 2 & 3). In August, many potential mosquito breeding sites were dry, while the September rains restored some of them with water. Within the surveys, inspection of rainwater barrels and manholes had priority, because they more frequently contain water, even during the dry summer period.

The survey was based on immature sampling (larvae and pupae) from breeding sites by means of a small net, a white plastic tray and plastic pipettes. *Aedes japonicus* was considered present when at least one larva of the species was observed in at least one water container at the site under consideration. The species was considered absent when it was not found in at least five potential favourable larval sites (and containing water for several days), of which at least three contained immatures of another mosquito species. Sites that have been surveyed and that did not have these characteristics (dry or mosquito-free sites) are not listed here.

A sample of immatures was systematically collected from each site for species identification. Initially the identification in the larval stage was carried out visually in the field; *Ae. japonicus* larvae can be distinguished relatively easily from *Culex* larvae with a simple pocket magnifier. Subsequently all specimens were confirmed under microscope in the laboratory. Collected pupae were reared to adults (females and males), which were then identified under microscope. Species identification was performed based on standard morphological keys (Schaffner et al. 2001, Schaffner et al. 2013a). Sub-samples are kept for genetic analysis and some

are deposited at the MNHNL Scientific Research Centre for reference.

## 2.2. Risk assessment

To assess the risk that this invasive mosquito species represents to biodiversity and its impacts on several targets, we performed a standard risk assessment applying two protocols developed by the Belgian Forum on Invasive Species (BFIS) and widely used in north-western Europe: ISEIA and Harmonia+.

The ISEIA protocol (Invasive Species Environmental Impact Assessment) enables an expert group to quickly evaluate the potential risk of a species; it addresses the dispersion potential and the environmental impacts of a species (Branquart 2009). The assessment contains four scores each with three levels (1=low, 2=medium, 3=high): a) dispersal potential, b) colonisation of high conservation value habitats, c) adverse impacts on native species, d) risk of alteration of ecosystem functions. Back in 2015, a first assessment of the East Asian bush mosquito was performed based on the ISEIA protocol under its synonym *Hulecoeteomyia japonica*. The resulting index value [C0] (scores 2+1+1+1) reflects that the assessors did not consider the species to be a threat for native biodiversity and ecosystems [C] and that it was absent from the area covered by the assessment [0] (Ries et al. 2017: 68). We performed an update.

Harmonia+ is a protocol compliant with criteria of the EU regulation (Anonymous 2014) for risk assessments for listing IAS of EU concern and considering, above environmental risks, further criteria like impacts on plants, animals and humans (including their health), impacts on human infrastructure, on ecosystem services and effects of climate change on these risks. As results are numerical scores between 0 and 1, they can only be interpreted when comparing different species or in the frame of a ranking scheme. This is why we chose to compare the East Asian bush mosquito to another invasive mosquito species from Asia, the Asian tiger mosquito *Ae. albopictus*, which we expect to appear in Luxembourg in the short term, as it is at present established in neighbouring regions of the Grand Duchy.

## 2.3. Biodiversity

In our field studies, mosquitoes were collected by immature sampling from aquatic breeding sites. Up to 106 sites out of 94 localities / 70 municipalities were inspected, focusing on water-holding containers (Fig. 2 & 3). At each location, several containers were checked and when detected, mosquito samples were systematically collected and identified. This allowed to detect the presence of other species than *Ae. japonicus* and to assess the mosquito species diversity.

## 3. Results

### 3.1. First record of *Aedes japonicus* in Luxembourg

The MNHNL molecular laboratory confirmed the initial morphospecies assignment by comparing the mitochondrial DNA sequences of the cytochrome c oxidase subunit I (COI) marker of three specimens with reference sequences deposited in the Barcode of Life Database under MNHNL001-19, MNHNL002-19, MNHNL003-19 (BOLD Systems, Ratnasingham & Hebert 2007).

### 3.2. Distribution of *Aedes japonicus* in Luxembourg

In a first mission, a quick field prospection on 1<sup>st</sup> and 2<sup>nd</sup> of August 2018 showed the Stolzembourg observation was not an isolated case. We could find the species in open water containers distributed along the whole rue du Faubourg where the species was first discovered (Fig. 2). As for the surrounding areas, we found the East Asian bush mosquito to be also present in Bivels, Vianden and Wahlhausen, as well as in Gemünd (DE).

Subsequently, a second field mission was entrusted in order to better assess the dispersal area of the species in Luxembourg, including a 3-day field study (14-16 August 2018) in concentric circles of about 10 km around Stolzembourg. This second mission confirmed the presence of *Ae. japonicus* over a large area estimated to cover at least 380 km square, located in the east of the Grand Duchy, from the middle Our valley in the North to Manternach in the South, and as



far as Kautenbach and Ettelbruck in the West. However, the boundaries of the colonised area could not be defined due to time constraints, particularly to the West and South, as the distribution area proved to be larger than initially expected. Consequently, a third mission was planned as a 5-day field study (1-5 October 2018) to complete the assessment of the colonised area.

In total, 106 sites out of 94 localities/70 municipalities were inspected, including 91 in Luxembourg (79 localities in 55 municipalities), 12 in Germany (including 3 sites monitored by German colleagues), 2 in Belgium and 1 in France (Table 1). In Luxembourg, *Ae. japonicus* was detected in 16 sites distributed over 12 localities, 12 municipalities, 7 cantons and 3 districts. The colonised area can be estimated to cover at least 550 km square by October 8th, 2018, located in the east of the Grand Duchy, from the middle Our valley in the North to Ernster in the South, and as far as Kautenbach and Ettelbruck in the West (Fig. 4, Table 1). The mosquito was not detected in neighbouring Belgium (Clairefontaine and Ouren) but in Germany, where it has been present for several years in Rhineland-Palatinate (Kampen H., personal communication) and from where it has most likely migrated to the Grand Duchy. We also detected the species in the French bordering village of Contz-les-Bains, which suggests that the mosquito is probably present, although not detected to date, in the region bordering the Moselle, from Manternach to the French border.

### 3.3. Risk assessments of *Aedes japonicus*

#### 3.3.1. ISEIA protocol

An update of this assessment on 13 August 2018 resulted in higher scores [C2] (scores 3+2+2+1), but left the species in the same category [C] (no threat for native biodiversity and ecosystems), with a regional distribution [2] (Ries & Pfeiffenschneider 2018). The four scores (1=low, 2=medium, 3=high) mean that the species shows [a=3] a high dispersal potential, [b=2] a medium risk of colonisation of high conservation value habitats, [c=2] a medium risk of adverse impacts on native species, and [d=1] a low risk of altera-

tion of ecosystem functions. Concerning the third score [c=2], there exists to date only one publication describing how larvae of *Ae. japonicus* are highly effective competitors and can reduce populations of native mosquito populations significantly through interspecific competition for limited resources. Surveys in Connecticut in 2005 revealed that *Ae. japonicus* was the dominant species collected in all waste tyres and natural rock pool environments. Comparisons with data from previous years indicate a significant decline of native species including *Ae. atropalpus*, *Ae. triseriatus* and *Cx. restuans* (Andreadis & Wolfe 2010). Since these results concern local populations occurring in natural rock pools that are present in the USA but not in Luxembourg, this led the assessors to give it a medium impact score.

#### 3.3.2. Harmonia+ protocol

The resulting scores of both assessment of *Ae. japonicus* and *Ae. albopictus* with the internet-based Harmonia+ protocol (D'Hondt et al. 2015, Vanderhoeven et al. 2015) are presented in Table 3.

Unfortunately, the Harmonia+ protocol calculates the overall invasion score as a geometric means by default, which means that if one invasion score equals 0, then the overall score equals 0 as well. This can happen with species like e.g. *Ae. albopictus* with very low active spread rates which are aggregated as 0 by the default Harmonia+ settings. This is why we preferred to use the arithmetic mean to calculate the overall invasion score (both mean values are presented in Table 2).

The overall invasion score for *Ae. japonicus* is quite high with 0.67 being the arithmetic mean of the scores for introduction, establishment and spread (geometric mean = 0.63). In comparison to this, the overall invasion score for *Ae. albopictus* is 0.47 (arithmetic) and respectively 0 (geometric). Indeed, while *Ae. japonicus* can actively colonise new areas with a speed of 10-20 km / year as long as the land cover provides the suitable corridors of forests, hedges or alleys, *Ae. albopictus* is very slow to actively spread by hitchhiking in vehicles.



Fig. 2. First example of potential larval breeding site, i.e. water barrels in a private garden parcel. Stolzembourg (49.962°N, 6.168°E), 1 August 2018, yielding *Aedes japonicus* and *Culex pipiens/torrentium*. Photo credit: Francis Schaffner.



Fig. 3. Second example of potential larval breeding site, i.e. road drain on public domain. Eisenbach (50.000°N, 6.145°E), 2 August 2018, yielding *Culex pipiens* and *Cx. torrentium*. Photo credit: Francis Schaffner.

While the environmental impact score (0.167) confirms the low environmental impact of *Ae. japonicus*, with *Ae. albopictus* having the same score, their impact on animals and humans appear significantly higher (with higher values for *Ae. albopictus*). As the overall impact score simply takes over the highest impact value, *Ae. albopictus* reveals a higher overall impact score ( $0.583 > 0.5$ ).

As for the overall risk score, it is calculated as the product of the overall invasion and impact scores. *Aedes japonicus* (0.335)

would rank in the middle range in a prioritisation scheme. Due to its lower overall invasion score, *Ae. albopictus* presents a significantly lower overall invasion score of 0.27.

### 3.4. Mosquito biodiversity

In addition to *Ae. japonicus*, eight mosquito species were observed in Luxembourg during these field studies (Tables 1 & 3), the most frequent being *Culex pipiens* and *Cx. torrentium* (89 sites). These 2 spe-

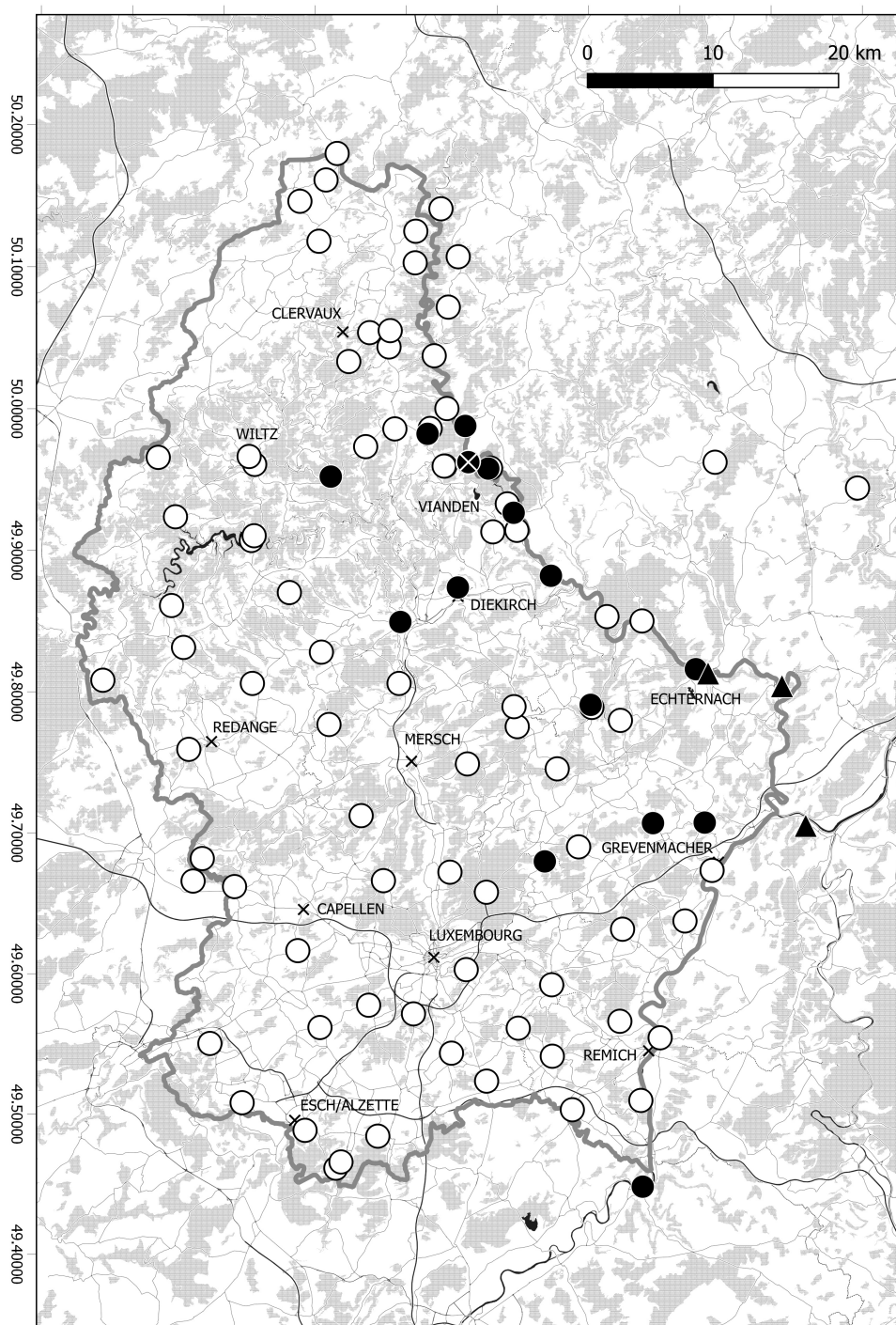


Fig. 4. Distribution of *Aedes japonicus* known as of 10 October 2018, for Luxembourg and the neighbouring regions of Belgium, France and Germany. White dots: undetected presence. Black dots: proven presence. Black dot with white X mark: first record in Stolzembourg. Black triangles: proven presence reported by D. Werner & H. Kampen (Germany).

Table 1. List of the 106 sites out of 94 localities/70 municipalities inspected in Luxembourg and bordering Germany, Belgium and France, between 1 August and 5 October 2018.

(I) Country / Region. M = Moselle (NUTS 3). A = Arlon (NUTS 2). L = Liège (NUTS 2). RP = Rhineland-Palatinate (NUTS 2). (II) Locality. (III) Latitude N (WGS 84). (IV) Longitude E (WGS 84). (V) Date. (VI) Status of *Aedes japonicus*. P = Present; species observed. A = Absent; species not observed. \* = data provided by D. Werner & H. Kampen. (VII) Other indigenous species observed. AeG = *Aedes geniculatus*, AeJ = *Ae. japonicus*, AnC = *Anopheles claviger* s.s., AnP = *An. plumbeus*, CxH = *Culex hortensis*, CxP/T = *Cx. pipiens* / *Cx. torrentium*, CxP = *Cx. pipiens*, CxT = *Cx. torrentium*, CsA = *Culiseta annulata*, CsL = *Cs. longiareolata*.

I	II	III	IV	V	VI	VII
Country/ region	Locality	Lat N	Lon E	Date	Status of <i>Aedes japonicus</i>	Other indigenous mos- quito species observed
LU	Arsdorf	49.861	5.843	01/10/2018	A	CxH, CxT
LU	Basbellain	50.146	5.983	01/10/2018	A	CxP/T
LU	Bavigne	49.924	5.847	01/10/2018	A	CxP/T
LU	Belvaux	49.508	5.920	03/10/2018	A	CxH, CxP/T, CsL
LU	Bettel	49.914	6.221	01/08/2018	A	CxP/T
LU	Beyren	49.632	6.337	04/10/2018	A	CxH, CxP, CsL
LU	Bivels	49.958	6.192	01/08/2018	A	CxP
LU	Bivels	49.958	6.190	01/08/2018	P	AeG, AnP, CxP/T
LU	Biwer	49.707	6.371	04/10/2018	P	CxH, CxP/T, CsA, CsL
LU	Blumenthal	49.745	6.266	15/08/2018	A	CxP/T
LU	Boevange-s.-Attert	49.777	6.015	02/10/2018	A	CxP/T
LU	Bollendorf-Pont	49.850	6.358	15/08/2018	A	CxP/T, CsA
LU	Bonnevoie	49.603	6.166	05/10/2018	A	AnP, CxP/T
LU	Bous	49.566	6.334	05/10/2018	A	CxH, CxP, CsA, CsL
LU	Colmar-Berg	49.806	6.092	02/10/2018	A	CxP/T
LU	Consdorf	49.780	6.335	15/08/2018	A	CxP/T
LU	Consthum	49.973	6.056	14/08/2018	A	CxP/T, CsL
LU	Dalheim	49.541	6.260	03/10/2018	A	CxP/T
LU	Diekirch	49.874	6.157	15/08/2018	P	CxP/T
LU	Dillingen	49.853	6.320	15/08/2018	A	CxP/T
LU	Dudelange	49.484	6.069	03/10/2018	A	CxP, CsL
LU	Echternach	49.816	6.418	15/08/2018	P	CxP/T
LU	Eischen	49.682	5.876	02/10/2018	A	CxP/T
LU	Eisenbach	50.000	6.145	02/08/2018	A	CxP, CxT
LU	Ell	49.759	5.862	01/10/2018	A	CxP/T
LU	Ernster	49.680	6.252	05/10/2018	P	CxP/T
LU	Ernzen	49.775	6.222	02/08/2018	A	CxP/T
LU	Esch-sur-Alzette	49.488	5.989	03/10/2018	A	AnP, CxH, CxP/T
LU	Esch-sur-Sûre	49.907	5.930	16/08/2018	A	AnC, CxP/T
LU	Esch-sur-Sûre	49.910	5.933	16/08/2018	A	CxT, AnP
LU	Ettelbruck	49.849	6.094	16/08/2018	P	CxP/T, CsL
LU	Fouhren	49.913	6.195	01/08/2018	A	CxP
LU	Frisange	49.523	6.188	04/10/2018	A	CxP, CsL
LU	Grevenmacher	49.673	6.435	04/10/2018	A	CxP/T, CsL
LU	Heisdorf	49.672	6.148	02/10/2018	A	CxP/T
LU	Hoesdorf	49.882	6.259	15/08/2018	P	CxP/T
LU	Hollenfels	49.712	6.051	02/10/2018	A	CxP/T
LU	Holzem	49.616	5.981	02/10/2018	A	CxP, CsA
LU	Holzthum	49.986	6.088	14/08/2018	A	CxP/T

LU	Huldange	50.161	6.012	01/10/2018	A	CxT
LU	Kalborn	50.103	6.110	14/08/2018	A	CxP/T
LU	Kautenbach	49.952	6.017	14/08/2018	P	CxP/T
LU	Kockelscheuer	49.571	6.108	03/10/2018	A	CxP/T
LU	Kopstal	49.666	6.075	02/10/2018	A	CxP/T
LU	Larochette	49.789	6.218	15/08/2018	A	CxP/T
LU	Lieler	50.125	6.110	14/08/2018	A	CxP/T
LU	Manternach	49.707	6.427	15/08/2018	P	CxP/T
LU	Marbourg	50.043	6.081	14/08/2018	A	CxP/T
LU	Marnach	50.054	6.060	14/08/2018	A	CxP/T
LU	Merscheid	49.870	5.972	02/10/2018	A	CxP/T
LU	Mertzig	49.828	6.007	02/10/2018	A	CxP, CsL
LU	Mondorf-les-Bains	49.503	6.282	04/10/2018	A	CxP/T
LU	Moutfort	49.592	6.260	05/10/2018	A	AnP, CxP/T
LU	Mullerthal	49.789	6.304	15/08/2018	A	CxP/T
LU	Mullerthal	49.791	6.302	15/08/2018	P	-
LU	Munshausen	50.033	6.037	14/08/2018	A	CxP/T
LU	Niederdonven	49.637	6.406	04/10/2018	A	CxH, CxP, CsA, CsL
LU	Perl�	49.808	5.767	01/10/2018	A	CxP, CsL
LU	P�tange	49.550	5.885	03/10/2018	A	CxP/T, CsL
LU	Pratz	49.806	5.931	02/10/2018	A	CxP/T
LU	Putscheid	49.960	6.142	02/08/2018	A	CxP
LU	Putscheid	49.959	6.142	02/08/2018	A	CxP, CxT
LU	Rambrouch	49.831	5.856	01/10/2018	A	CxP/T, CsA
LU	Reckange-s.-Mess	49.562	6.005	03/10/2018	A	CxP/T, CsL
LU	Remich	49.554	6.378	04/10/2018	A	CxP
LU	Rodenbourg	49.690	6.289	04/10/2018	A	CxP/T
LU	Roder	50.055	6.082	14/08/2018	A	CxT, CsL
LU	Rodershausen	50.037	6.131	02/08/2018	A	CxT
LU	Roeser	49.543	6.150	03/10/2018	A	CxP/T
LU	Rumelange	49.461	6.023	03/10/2018	A	CxP, CsL
LU	Rumelange	49.466	6.028	03/10/2018	A	CxH, CxP/T, CsL
LU	Schlewenhof	49.577	6.059	03/10/2018	A	CxP/T
LU	Schm�tt	50.180	6.024	01/10/2018	A	CxP/T
LU	Schoos	49.749	6.167	02/10/2018	A	AnP, CxT, CsL
LU	Schwebsange	49.510	6.357	04/10/2018	A	CxP/T, CsL
LU	Sonlez	49.966	5.828	01/10/2018	A	AnP, CxT
LU	Steinfort	49.662	5.912	02/10/2018	A	CxP/T, CsL
LU	Stolzembourg	49.962	6.169	04/07/2018	P	n/a
LU	Stolzembourg	49.962	6.168	01/08/2018	P	CxP/T
LU	Stolzembourg	49.962	6.168	01/08/2018	P	CxP
LU	Stolzembourg	49.962	6.167	01/08/2018	P	CxP/T
LU	Stolzembourg	49.963	6.169	01/08/2018	P	CxP/T
LU	Syren	49.561	6.223	03/10/2018	A	CxP/T
LU	Troisvierges	50.118	6.004	01/10/2018	A	CxP/T
LU	Vianden	49.933	6.211	01/08/2018	A	CxH, CxP/T
LU	Vianden	49.927	6.218	01/08/2018	P	AnP, CxP/T
LU	Wahlhausen	49.986	6.126	02/08/2018	A	CxP/T
LU	Wahlhausen	49.982	6.123	02/08/2018	P	CxP/T
LU	Waldhof	49.658	6.188	05/10/2018	A	CxP/T
LU	Wiltz	49.961	5.934	14/08/2018	A	CxP/T, CsA
LU	Wiltz	49.966	5.928	14/08/2018	A	CxP, CsA
FR/M	Contz-les-Bains	49.448	6.359	04/10/2018	P	CxH, CxP/T, CsL

BE/A	Clairefontaine	49.666	5.866	02/10/2018	A	AnC, CxH
BE/L	Ouren	50.141	6.138	14/08/2018	A	CxP/T
DE/RP	Dahlen	50.072	6.146	14/08/2018	A	CxP/T
DE/RP	Echternacherbrück	49.813	6.431	31/08/2018	P*	n/a
DE/RP	Gemünd	49.988	6.165	02/08/2018	P	CxP/T, AnP
DE/RP	Heidweiler	49.910	6.744	13/08/2018	P	CxP/T
DE/RP	Klausen	49.904	6.883	13/08/2018	A	CxP/T
DE/RP	Morbach	49.818	7.132	13/08/2018	P	CxP/T, AnP
DE/RP	Oberweis	49.962	6.439	15/08/2018	A	CxP/T
DE/RP	Ralingen	49.804	6.512	31/08/2018	P*	n/a
DE/RP	Röhl	49.944	6.595	15/08/2018	A	CxP/T
DE/RP	Sevenig	50.107	6.157	14/08/2018	A	AnP
DE/RP	Simmern	49.981	7.511	13/08/2018	P	CxP/T
DE/RP	Wasserliesch	49.705	6.538	01/09/2018	P*	n/a

Table 2. Risk assessments of *Aedes japonicus* and *Ae. albopictus* for Luxembourg according to the Harmonia+ protocol. Default operations used, apart from overall invasion score calculated as arithmetic mean instead of geometric. All module and question weights considered equal.

	Module	<i>Aedes japonicus</i>	<i>Aedes albopictus</i>	Aggregation method
Invasion	Introduction score	0.5	0.667	arithmetic
	Establishment score	1.0	0.75	arithmetic
	Spread score	0.5	0.0	arithmetic
	(Overall invasion score)	(0.63)	(0.0)	(geometric)
	Overall invasion score	0.67	0.47	arithmetic
Impacts	Environmental impact score	0.167	0.167	arithmetic
	Plant impact score	0.0	0.0	arithmetic
	Animal impact score	0.333	0.5	arithmetic
	Human impact score	0.5	0.583	arithmetic
	Other impact score	0.0	0.0	arithmetic
	Overall impact score	0.5	0.583	maximum
	Overall risk score	0.335	0.27	product

cies cannot be distinguished at the larval stage (hence the joint indication *Cx. pipiens* / *torrentium*) but can be distinguished at the adult stage, male in particular, obtained from immatures, which was done in 25 cases, with a result of 17 occurrences for *Cx. pipiens* and 9 for *Cx. torrentium*, the species being found sympatric in only one sample. The other species include species commonly found in artificial habitats (*Culiseta annulata*, *Cs. longiareolata*, *Cx. hortensis*, 8, 21, and 8 sites, respectively) or in tree holes (*Ae. geniculatus*, *Anopheles plumbeus*, 1 and 8 sites, respectively); the most rarely observed species is restricted to ponds and ditches (*An. claviger*, 1 site). Among these, two species are new to the fauna of the

Grand Duchy, namely *Cx. hortensis* and *Cs. longiareolata*.

Overall, among the 91 sites revealing mosquitoes, most of them contained 2 or 3 sympatric species (n=53; 58.2% and n=27; 29.7%, respectively), whereas few harboured 4 or 5 species (n=6; 6.6% and n=3, 3.3%) or only 1 species (n=2, 2.2%).

#### 4. Discussion

Here we report the actions upon the detection of the invasive mosquito species *Ae. japonicus* in Luxembourg, subsequent to a nuisance report by a citizen, from a village located in the Our valley, at the north-east

Table 3. Occurrence of mosquito species and their larval breeding sites observed in Luxembourg between 1 August and 5 October 2018.

Mosquito species	No. of positive sites	Observed main type of larval breeding sites per site and numbers
<i>Aedes geniculatus</i>	1	Rain water barrel
<i>Aedes japonicus</i>	16	Road drains (7), tyres (2), barrels and other containers (5), tarpaulin (1), stone fountain (1)
<i>Anopheles claviger</i> s.s.	1	Road drains
<i>Anopheles plumbeus</i>	8	Plastic and wooden barrels (4), tyres (2), tarpaulin (1), road drains (1)
<i>Culex hortensis</i>	8	Barrels and other containers (5), road drains (3), stone basin (1)
<i>Culex pipiens</i> / <i>Cx. torrentium</i>	89	Road drains (48), barrels and other containers (24), tyres (11), tarpaulin (4), concrete basin (2)
<i>Culiseta annulata</i>	8	Road drains (3), barrels and other containers (3), tyres (1), basin (1)
<i>Culiseta longiareolata</i>	21	Road drains (13), barrels and other containers (6), tyres (2)

border to Germany. Three mosquito females were sent to the MNHNL because of their unusual appearance, i.e. being larger and differing in colour compared to the common brownish house mosquito (*Cx. pipiens*), bearing clear white stripes on a black background on legs and abdomen (Fig. 1). These females were also collected because of their human biting behaviour, in and around the citizen home. Based on our results (Schaffner 2018), the presence of this invasive mosquito species was immediately communicated to local authorities and to the public via the media and the Museum’s neobiota.lu website (Ministère de la Santé, 2018, Ries & Pfeiffenschneider 2018), with suggested measures to reduce mosquito breeding around human dwellings.

The field studies demonstrate the establishment of *Ae. japonicus* over at least 550 km<sup>2</sup> (21% of the country land), in the eastern part of the country. The citizen reported that mosquito being observed for the first year, but considering the large colonised area, we estimate the species has been present but unnoticed in Luxembourg for more than one year. Confirmed presence of this species in bordering France along the Moselle suggests its occurrence over a larger territory, in particular in the southeast region. Recently established populations could have remained unnoticed by us since the dry summer conditions did not facilitate the

detection of larvae, with numerous potential larval breeding sites (e.g. tyres, tree holes) being dried out during our survey. Considering the presence of the species in Rhineland-Palatinate up to the border to Luxembourg, we can assume the species has spread from Germany into the Grand Duchy. Luxembourg is now the thirteenth European country facing its invasion. Considering the presence of numerous suitable larval habitats (natural and man-made containers) and favourable environment (forested corridors), we can assume the species will spread further throughout the country towards north, west and south. According to the spread observed in Switzerland (Schaffner, personal observations), i.e. 10-20 km/year, the Grand Duchy territory might be fully colonised by end of 2020.

Invasive species represent a risk to biodiversity by outcompeting native species, but mosquitoes may also generate risk to human and animal health by transmitting pathogens (Versteirt & D’hondt, 2013). As shown by the performed risk assessments, *Ae. japonicus* is considered to have a relatively low impact on the environment, while it presents quite a high overall invasion score and a middle field impact score. Compared to this, *Ae. albopictus* is spreading less fast, has a similarly low impact on the environment, but has a higher overall impact score due to the fact that *Ae. albopictus* is much more



impacting on human and animal health. *Aedes japonicus* generates nuisance during daylight and especially at sunset, but only under particular conditions, namely in and near to forested areas (Medlock et al. 2015, Kampen & Werner 2014). In our study as well, the first report is connected to nuisance at a house bordering a forest, and no biting females were observed during our daylight survey at colonised sites.

In our survey, nine mosquito species were collected, which is a significant result compared to the 15 species that constitute the local mosquito fauna before our study (Beck et al. 2003, Robert et al. 2019). This result is particularly relevant considering that only man-made containers were sampled, with 88% of the samples showing 2 or 3 mosquito species being present at a single site (but not necessarily in a single container, since several containers were inspected at each site). Most of the observed species are known to breed in such habitats, but for other species this represents unusual behaviour. Natural containers (i.e. tree holes) did not contain water during our survey and other natural habitats (e.g. ponds and ditches) were not investigated at all. The presence of *An. claviger* in man-made containers is quite rare but it has been observed elsewhere, whereas the collection of *Ae. geniculatus* and *An. plumbeus* in such larval habitats is not uncommon, in particular when dead leaves are decaying in the container (Schaffner 2003). Man-made containers are the favoured larval habitat for *Cx. hortensis* and *Cs. annulata*, the latest being frequently observed to breed in ponds (Schaffner et al 2001). This is also the case for both *Cx. pipiens* and *Cx. torrentium*, which were collected mainly from road drains but also other containers. They are the most abundant mosquito species in rural and urban environments according to our study. They preferably bite birds, but *Cx. pipiens* (the common house mosquito) originating from sewage and groundwater (sewers, pits) bites mammals, including humans, at night. Finally, besides the confirmation of the presence of *Ae. japonicus*, two species new to the fauna of Luxembourg have been detected. The first, *Cx. hortensis*, is native to Benelux but was not collected in the Grand Duchy by former studies (Beck et al. 2003). However,

the species was previously observed for the first time in 2001 based on larval sampling (Schaffner F., unpublished data). The second species, *Cs. longiareolata*, has a rather southern classical distribution but seems to have gradually colonised more northern regions of Europe in recent years (France: Schaffner F., personal observations; Germany: Kampen et al. 2017; Switzerland: Schaffner et al. 2009). Females of both species are preferably biting birds and had never (*Cx. hortensis*) or very rarely (*Cs. longiareolata*) been observed to bite humans, and therefore do not represent any significant threat to human health (Schaffner et al. 2001). While *Cx. hortensis* is most likely a native species, the collection of *Cs. longiareolata* from several locations distributed over the Grand Duchy suggests its presence for several years.

In summary, two mosquito species are added to the known fauna of Luxembourg, which reaches now the number of 18 species. Both species might soon be present all over the Grand Duchy. While specifically searching for *Ae. japonicus*, the finding of another mosquito species new to the country demonstrates the local mosquito fauna is being still poorly known. This, and considering the fauna of neighbouring regions/countries and the current spread of invasive mosquitoes, in particular *Ae. albopictus*, pleads for further in-depth field studies to be performed to better describe the mosquito fauna of the Grand Duchy.

## 5. Conclusions

Our field studies reveal a dispersion of the alien invasive mosquito *Ae. japonicus* in Luxembourg over a large area. This, coupled with the fact that the species colonises both natural (tree holes scattered in forests) and artificial (all kinds of man-made containers) water containers, many around dwellings and often inaccessible (private property), makes the objective of eliminating the population unrealistic. The persistence and spread of the species on the Grand Duchy territory also seems unavoidable given the local environment and climate, particularly along forested corridors, and in all direc-



tions (populations also exist in all neighbouring countries).

The vector risk represented by the species is limited, but not zero. Human and animal health intervention can therefore only consist of risk management, namely (i) population monitoring (for presence and abundance) and (ii) keeping the population below a certain threshold in order to prevent or limit nuisance and vectorial risk.

In addition, the discovery of a second species new to the mosquito fauna of the Grand Duchy demonstrates the current limits of knowledge of this fauna and consequently of the related vectorial risk. Therefore the authors propose possible future actions in the field of (a) surveillance, (b) outreach, (c) treatment of invasive mosquito breeding sites and (d) collection of baseline data:

(a) surveillance of invasive mosquitoes, including the *Ae. albopictus* tiger mosquito, to detect and assess their presence and abundance, using ovitraps on a selection of high-risk sites (motorway rest areas, freight transport platforms, international coach line bus stations, airports, etc.), and to take appropriate preventive and curative measures during high-risk periods; joint surveillance of arboviruses (West Nile, dengue, and chikungunya viruses in particular) is also recommended for the prevention of local transmissions, in addition to entomological surveillance;

(b) outreach through communication with the general public, in order to disseminate basic preventive recommendations, namely to limit the proliferation of mosquitoes around dwellings by controlling / eliminating mosquito breeding sites and protecting itself from bites;

(c) treatment of productive invasive mosquito breeding sites on public property with suitable biological biocide (combined active substances *Bacillus thuringiensis israelensis* ser. H14 and *Lysinibacillus sphaericus*); however, this requires prior authorisation for the import and use of these substances and training of staff in their application; monitoring should make it possible to assess the productivity of the different types of colo-

nised breeding sites in order to define treatment priorities;

(d) collection of baseline data by producing an atlas of mosquitoes of Luxembourg based on field studies which could facilitate the detection of new populations of invasive mosquitoes, provide a complete picture of the mosquito fauna of the Grand Duchy, and better assess the risks induced by the presence of potentially vector species of parasites and arboviruses.

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