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SHORT COMMUNICATION

First record and spreading of the invasive mosquito *Aedes japonicus japonicus* (Theobald, 1901) in Croatia

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Abstract. *Aedes* (*Hulecoeteomyia*) *japonicus japonicus* (Theobald, 1901) has recently established across North America and Central Europe. A 3-year survey was conducted in northwestern Croatian regions from 2013 to 2015 using mosquito ovitraps at possible points of entry and house yards, occasionally complemented by larval collections from cemetery vases. In the first year, the survey investigated the county bordering Slovenia, where the first detection of *Ae. j. japonicus* had taken place on 28 August 2013. During the next 2 years, *Ae. j. japonicus* was detected in this area from early May until late October. In 2015, several counties further to the east were included in the survey, leading to the detection of *Ae. j. japonicus* approximately 100 km eastward from the initially surveyed region. Given a moderate continental climate and homogeneous climatic conditions in this part of Europe, the eastward spread of *Ae. j. japonicus* can be expected to continue.

Key words. *Aedes j. japonicus*, Croatia, first record, surveillance, mosquito spreading.

Aedes (*Hulecoeteomyia*) *japonicus japonicus* (Theobald, 1901) (Diptera: Culicidae) (taxonomic nomenclature *sensu* Wilkerson *et al.*, 2015), also known as the Asian bush or rock pool mosquito, is an invasive mosquito species that originates from East Asia and the Far East (Tanaka *et al.*, 1979). Initial detections outside the native range of this species were reported in 1993 from New Zealand (not established) and 1998 from the northwestern U.S.A., where it rapidly spread throughout the eastern and northern states and invaded southern Canada (Kampen & Werner, 2014; Kaufman & Fonseca, 2014). In Europe, the species was detected for the first time in 2000 in a storage yard of imported used tyres in northwestern France, where it was promptly eliminated (Schaffner *et al.*, 2009). To date, *Ae. j. japonicus* has established in Belgium, Switzerland, Germany, the Netherlands, Austria and Slovenia (Kampen & Werner, 2014; Kaufman & Fonseca, 2014; Ibáñez-Justicia *et al.*, 2014), as well as more recently in Hungary, Liechtenstein and Italy (Seidel *et al.*, 2016a, 2016b).

Aedes j. japonicus is not considered to be a major vector of human pathogens, and its role in transmission under natural conditions remains unclear. Laboratory studies showed its high vector competence for several arboviruses, such as Japanese encephalitis virus (JEV), West Nile virus (WNV), La Crosse encephalitis virus (LACV) and dengue virus (DENV). In addition, this species is a moderately efficient vector of chikungunya (CHIKV), eastern equine encephalitis (EEEV) and St Louis encephalitis (SLEV) viruses (Schaffner *et al.*, 2013b).

Aedes j. japonicus is a cold-tolerant species, breeding in water with a high level of organic matter in diverse natural and artificial breeding sites. The species has a preference for forested areas (Kaufman & Fonseca, 2014).

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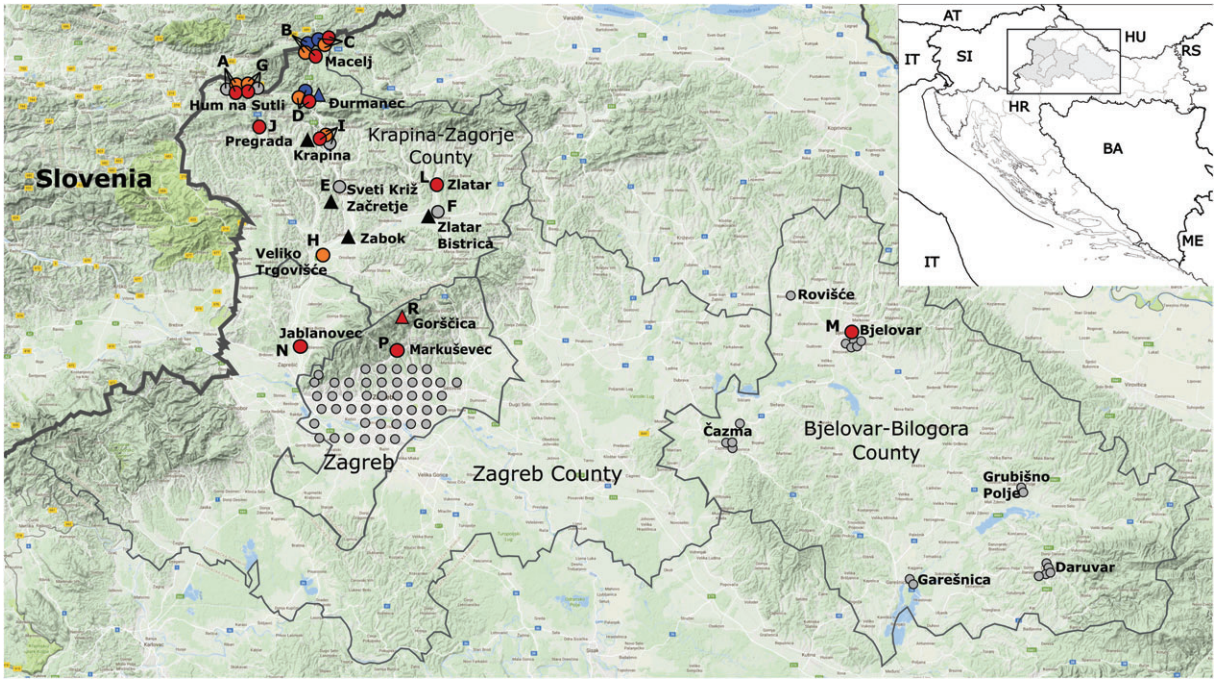

Fig. 1. Distribution of sampling locations from the 2013–2015 mosquito survey in northwestern Croatia. Coloured circles indicate locations where *Aedes japonicus japonicus* was detected and grey circles denote sampling locations where *Ae. j. japonicus* was not found. Positive locations are indicated by blue, orange and red circles for 2013, 2014 and 2015, respectively. Black triangles indicate positive cemeteries in the 2015 larval survey, and blue and red triangles mark locations with larvae other than cemeteries in 2013 and 2015, respectively. Abbreviation letters appearing within figure are explained in Table 1.

A 3-year mosquito survey was conducted in northwestern Croatia (Fig. 1) with the aim of investigating the presence/absence of *Ae. j. japonicus*. Northwestern Croatia is characterized by a moderate continental climate with hot summers and without extremely dry periods. The mean annual precipitation is 800–900 mm, with a minimum in winter (approximately 50 mm in February) and a maximum in summer (approximately 100 mm in August). The average daily temperature varies between 20 and 25 °C during the summer months and several degrees centigrade below freezing level during the winter (Zaninović *et al.*, 2008). These climate conditions are highly similar to those occurring in Hungary, Austria and Slovenia where *Ae. j. japonicus* has already been reported (Seidel *et al.*, 2012).

In 2013, the survey was carried out from July until October (calendar weeks 27 to 41) in Krapina-Zagorje County, a region bordering Slovenia (Fig. 1). In 2014, the surveillance was carried out during weeks 26–42 in the same county. In 2015, the survey was already started in week 21 and lasted until week 42. The area under surveillance was extended to the east by including Bjelovar-Bilogora County and Zagreb County (Fig. 1). In addition, the metropolitan area of Zagreb was under surveillance during weeks 21–41 in all three study years. Indeed, the City of Zagreb had been under mosquito surveillance for an even longer period, which was intensified using ovitraps after the detection of *Aedes albopictus* in 2004 (Klobučar *et al.*, 2006).

In 2013, surveillance in Krapina-Zagorje County was conducted at seven locations (Fig. 1 and Table 1), six of which represented possible entry points of invasive mosquito species (Schaffner *et al.*, 2013a). Every location included three ovitraps positioned 30–50 metres apart. Multiple ovitraps within a single location are known to improve the success of mosquito detection (ECDC, 2012). The selected locations included two on the border crossing between Croatia and Slovenia (locations B and C in Fig. 1), a cemetery (D), a petrol station on the highway (E), a tyre storage area (F), a truck parking area (G) and a house yard (A). In 2014, eight locations were surveyed: six of the 2013 locations (access was no longer granted to location F) and two new ones: another tyre repair shop (H) and a cemetery (I). The surveillance network in 2015 (Fig. 1) consisted of 10 locations, including seven from 2014 (except location H). Three new locations comprised a new tyre repair shop (J), a toll pay station on the highway (K) and a house yard (L).

In addition, larval collections from flower vases and other water-filled containers were performed in four cemeteries in Krapina-Zagorje County in 2015 (Fig. 1). The 2015 surveillance also included 20 locations (cemeteries, tyre repair shop, parking areas for trucks and petrol stations) in Bjelovar-Bilogora County. The only surveillance location in Zagreb County was a house yard (N in Fig. 1).

In the City of Zagreb, the monitoring was conducted at 57 locations with one ovitrap per location in 2013, 29 locations with three ovitraps per location in 2014 and 15 locations with three ovitraps per location in 2015 (Fig. 1).

The ovitraps were represented by 500 mL black plastic containers filled with tap water (approximately 300 mL) and a wooden slat (16 × 2.5 × 0.4 cm) as an oviposition substrate. Each

Table 1. Locations of *Aedes japonicus japonicus* egg and larval sampling, numbers of positive ovitraps/containers, average numbers of eggs and SD per ovitrap in Krapina-Zagorje County in the 2013–2015 surveillance seasons.

Location	Location label	Coordinates	Altitude m a.s.l.	Number of *Ae. j. japonicus* findings per year (%)	Average no. eggs per pos. ovitrap (SD)	Number of *Ae. j. japonicus* findings per year (%)	Average no. eggs per pos. ovitrap (SD)	Number of *Ae. j. japonicus* findings per year (%)	Average no. eggs per pos. ovitrap (SD)
				2013		2014		2015	
Hum na Sutli (A)	A1	46.17437, 15.62927	209	0	0	1†(5.9)	6	11†(50.0)	78 (91)
	A2			0	0	1†(5.9)	6	4†(18.2)	75 (68))
	A3			1‡		3†(17.6)	84 (64)	8†(36.4)	99 (90)
Macelj (B)	B1	46.27122, 15.86716	392	0	0	2†(11.8)	44	10†(47.6)	206 (265)
	B2			1 (8.3)†, 1‡	23	4†(23.5)	27 (16)	14†(63.6)	349 (344)
	B3			1‡		6†(35.3)	103 (60)	17†(77.3)	266 (225)
Macelj (C)	C1	46.27122, 15.86716	392	2‡		7†(41.2)	57 (35)	12†(54.5)	216 (221)
	C2			2‡		15†(88.2)	158 (176)	18†(81.8)	205 (192)
	C3			1 (8.3)†	17	13†(76.5)	67 (58)	15†(68.2)	72 (62)
Đurmanec (D)	D1	46.1993, 15.83864	215	0	0	2†(11.8)	95	6†(27.3)	107 (57)
	D2			0	0	1†(5.9)	196	7†(33.3)	194 (103)
	D3			1 (8.3)†, 1‡	65	1†(5.9)	47	1†(4.5)	44
Sv. Križ Začretje (E)	E1	46.06795, 15.91894	164	0	0	1‡		0	0
	E2			0	0	0	0	0	0
	E3			0	0	0	0	0	0
Zlatar Bistrica (F)	F1	46.04785, 16.0847	167	0	0	NS	NS	NS	NS
	F2			0	0				
	F3			0	0				
Hum na Sutli (G)	G1	46.22142, 15.70659	287	0	0	1†(5.9)	14	1†(4.5)	24
	G2			0	0	0	0	0	0
	G3			0	0	1†(5.9)	43	1†(4.8)	131
Veliko Trgovišće (H)	H1	46.00858, 15.85292	145	NS	NS	1†(6.7)	67	NS	NS
	H2					0	0		
	H3					0	0		
Krapina (I)	I1	46.16281, 15.87676	178	NS	NS	0	0	1‡	
	I2					2†(11.8)	40	3†(15.0)	43 (39)
	I3					1‡		0	0
Pregrada (J)	J1	46.15929, 15.75558	185	NS	NS	NS	NS	2†(9.1)	115
	J2							4†(19.0)	84 (68)
	J3							12†(54.5)	117 (105)
Krapina (K)	K1	46.13349, 15.88326	170	NS	NS	NS	NS	0	0
	K2							0	0
	K3							0	0
Zlatar (L)	L1	46.09541, 16.07632	179	NS	NS	NS	NS	2†(9.1)	27
	L2							1†(4.5)	121
	L3							1†(4.5)	14
Krapina§	▲	46.16281, 15.87676	178	NS	NS	NS	NS	1 (0.7)	
Zabok§	▲	46.02986, 15.912	152	NS	NS	NS	NS	1 (1.4)	
Zlatar Bistrica§	▲	46.05882, 16.06651	181	NS	NS	NS	NS	2 (2.1)	
Sv. Križ Začretje§	▲	46.07977, 15.91886	168	NS	NS	NS	NS	8 (11.1)	

†*Aedes j. japonicus* identified morphologically from reared material.
‡Species not identified.
§Locations of larval sampling.
NS – not sampled; pos. ovitrap – ovitrap with *Ae. j. japonicus* eggs.
Locations: A, L – house yards (sites on the location A); B – border crossing, entrance for cars to Croatia; C – border crossing, exit for trucks from Croatia; D – cemetery; E – petrol station; F – tyre storage area; G – parking area for trucks; H, J – tyre repair shops; I – cemetery; K – toll station on highway.

ovitrap was continuously exposed at the selected sites. The ovitraps were placed on the ground, hidden at the bases of trees or under vegetation. The wooden slats and water were replaced on a weekly basis. Upon collection, the slats were delivered to the laboratory. The eggs were counted using a stereo microscope (100× magnification). The slats with eggs were then maintained for 1 week in the laboratory environment (daylight exposure at 20–27 °C and 50–60% relative humidity, with no air-conditioning), aiming to ensure complete embryonic development and to allow hatching. The larvae were reared in the same kind of plastic containers used for the ovitraps filled with tap water. After hatching, the fourth-instar larvae were identified morphologically (ECDC, 2012).

A summary of the survey performed in Krapina-Zagorje County is provided in Table 1. The surveillance of 12 locations in the county made use of 36 ovitraps, from which 1269 wooden slats were inspected. On average, 106 slats were exposed per location (i.e. 35 slats per site).

Eggs of *Ae. j. japonicus* were first recorded in the cemetery in Đurmanec (D in Fig. 1) on 30 August 2013.

Subsequent to the first finding, an enhanced surveillance of location D was carried out in September 2013, and larvae of *Ae. j. japonicus* were collected from several vases and a water fountain. In September 2013, *Ae. j. japonicus* was also observed in Macelj at two locations at the border crossing with Slovenia: the entrance point to Croatia for personal cars (B) and the exit point from Croatia for trucks (C) (Fig. 1 and Table 1).

Two adult specimens of *Ae. j. japonicus* reared from eggs sampled at localities B and D were analysed genetically. This investigation revealed the microsatellite signature of genotype 1 (populations from South Germany, Switzerland and Slovenia) with a little admixture of genotype 2 (North and West German populations) (Zielke *et al.*, 2015).

In 2014, *Ae. j. japonicus* was detected at the same three locations from 2013 (B, C and D) and at several new locations. New findings included a private house yard (A) and a truck parking area (G), a tyre repair shop (H) and a cemetery (I) (Fig. 1 and Table 1).

Another two locations with *Ae. j. japonicus* were recorded in 2015: a tyre repair shop (J) and a house yard (L) (Fig. 1 and Table 1).

From three inspections in 2014, eggs of both *Ae. j. japonicus* and *Ae. albopictus* were collected on the same slat at location B, whereas, in 2015, a slat from location A contained eggs of both *Ae. j. japonicus* and *Aedes geniculatus*.

In total, 30 722 eggs of *Ae. j. japonicus* were collected during the 3-year field work in Krapina-Zagorje County. As can seen from Table 1, the numbers of eggs varied greatly between locations and years. In 2013, a total of 105 eggs of *Ae. j. japonicus* was detected on three slats (mosquito species from eight slats could not be identified), corresponding to 1.3% of all slats in 2013. The total number of *Ae. j. japonicus* eggs found on the slats in 2014 was 5347. Compared with 2013, a higher number of *Ae. j. japonicus* was recorded at the border crossing area at locations B and C in 2014 (Fig. 2 and Table 1). The first eggs were already recorded in the first observation week (week 26, June) at locations A and C. Another increase in the number of mosquito eggs occurred in 2015 when a total of 25 270 *Ae. j. japonicus* eggs was identified. Almost 75% of the eggs were collected from locations B and C. The earliest slat collection and identification of mosquito eggs in 2015 was undertaken in week 21 (the second half of May) (Fig. 2), when four out of 30 slats (approximately 13%) contained mosquito eggs.

The number of eggs increased during the course of the summer in both 2014 and 2015, with a maximum occurring in early

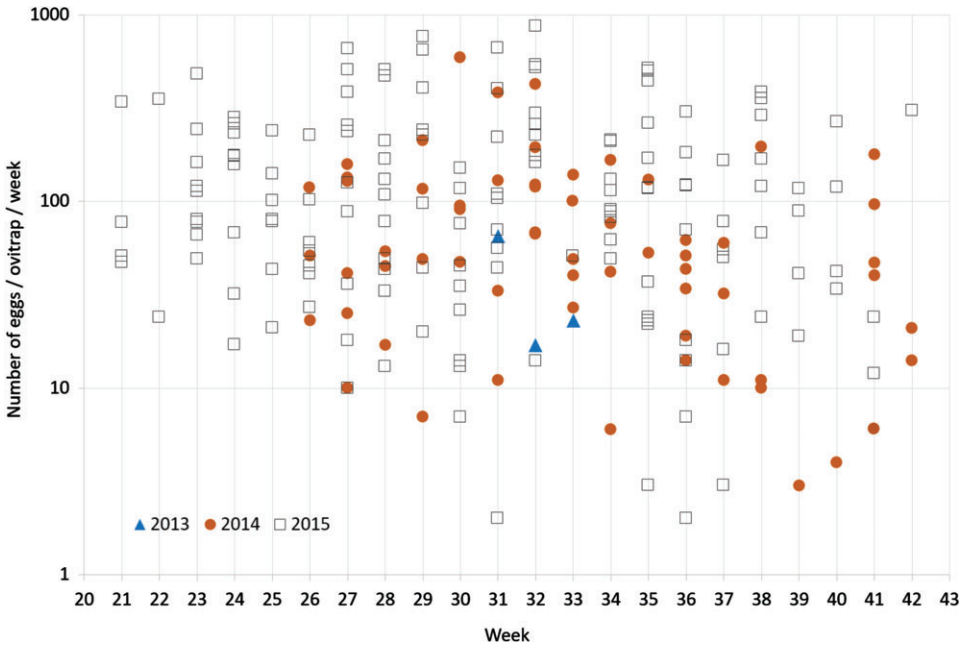

Fig. 2. Number of *Aedes japonicus japonicus* eggs per ovitrap per week from 2013 to 2015 in Krapina-Zagorje County. Y axis is logarithmic.

Table 2. Locations positive for *Aedes japonicus japonicus* egg sampling, numbers of positive ovitraps and average numbers of eggs in Bjelovar-Bilogora County, Zagreb County and the City of Zagreb in the 2015 surveillance season.

Location	Location label	Coordinates	Altitude, m a.s.l.	Number of *Aedes j. japonicus* findings per year (%)	Average number eggs per pos. ovitrap
Bjelovar†(M)	M1	45.90592, 16.85945	128	0	0
	M2			1 (4.5)	5
	M3			0	0
Jablanovec‡(N)	N1	45.87859, 15.84706	140	1 (4.5)	88
	N2			2 (9.1)	14
	N3			1 (4.5)	5
Markuševec§(P)	P1	45.89443, 16.02869	256	2 (100)	69
	P2			0	0
	P3			0	0

†Data from one positive location out of 20 locations sampled in Bjelovar-Bilogora County.
‡Location in Zagreb County.
§Location in the City of Zagreb.
pos. ovitrap – ovitrap with *Ae. j. japonicus* eggs.
Locations: M – tyre repair shop; N and P – house yards.

August (week 32) (Fig. 2). The absolute maximum of eggs was 1518, as recorded on a single slat collected on 11 August 2015 at location B. By contrast with 2013 with a single slat each with *Ae. j. japonicus* eggs in August and September, both 2014 and 2015 produced positive slats of *Ae. j. japonicus* in the second half of October (week 42) (Fig. 2).

A detailed survey of the cemeteries in Krapina-Zagorje County in August 2015 included an inspection of 369 vases filled with water (Table 1). Less than 4% of vases contained mosquito larvae. *Aedes j. japonicus* was detected in 12 out of 14 vases containing larvae (two vases contained *Culex pipiens* complex larvae only).

Subsequent to the increase of positive ovitraps and the number of mosquito eggs in Krapina-Zagorje County between 2013 and 2014 (indicating the establishment of the species), we expected *Ae. j. japonicus* to spread eastward. Therefore, the 2015 survey was extended to Bjelovar-Bilogora County (Fig. 1 and Table 2). In total, 60 ovitraps were installed at 20 locations, with each location consisting of three collection sites (one ovitrap/site). The survey included 22 weekly visits during which 1296 slats were inspected for the presence of the eggs (24 slats were not found during the inspection). Nine slats were positive for mosquito eggs, although *Ae. j. japonicus* was only observed on one slat collected in July from a tyre repair shop in Bjelovar (location M) (Fig. 1 and Table 2). Because this was the first record of *Ae. j. japonicus* in the county, a detailed investigation of the tyres in the shop was conducted, although no further mosquito larvae were observed.

During the surveillance in Jablanovec (N), Zagreb County, in 2015, 66 slats from three ovitraps were inspected (Table 2). Eggs of *Ae. j. japonicus* were recorded at four occasions in August and September. Despite intense ovitrap surveillance in the City of Zagreb that had started in 2004, no *Ae. j. japonicus* were detected before 2015. During the 2013–2015 period, 3357 slats were checked. *Aedes j. japonicus* was recorded for the first time in August 2015 at two sites on the northern outskirts of Zagreb. Larvae were collected from a wooden container next to the mountain cottage Gorščica (R) at 696 m a.s.l. (45.92902, 16.02924) and eggs at a house yard in the well-forested city suburb Markuševec (P) (Fig. 1 and Table 2).

During the whole study, mosquito eggs were recorded at six locations (A and D in 2013, E and H in 2014, M and P in 2015) in only one trap site each, which indicates the advantage of using multiple ovitraps within a single location, increasing the sensitivity for detection of invasive mosquito species (Tables 1 and 2).

The main finding of the survey is that *Ae. j. japonicus*, which was not known in this part of Central Europe until several years ago, has become well-established across northwestern Croatia. The reported results of a 3-year survey include the first record of *Ae. j. japonicus* in Croatia in August 2013 near the border with Slovenia. Over the next 2 years, *Ae. j. japonicus* developed in the bordering region in numbers that enabled its detection already in early May and still in October. Furthermore, in 2015, *Ae. j. japonicus* was detected approximately 100 km eastward from the initial 2013 detection location. The eastward spread of this species is proposed to have crossed Austria, Slovenia and northern Croatia and continued further east. This eastern part of Central Europe is characterized by climatic conditions assumed to support a further spread of *Ae. j. japonicus* to the east.

To date, our survey has not recognized *Ae. j. japonicus* as an aggressive human biter and the public health institutes in charge of the survey have not received complaints from citizens. This finding is in concordance with the findings of Kaufman & Fonseca (2014) who do not consider the species to be highly anthropophilic.

A continued surveillance of this species should ensure the timely implementation of mosquito control measures. An increased awareness of citizens regarding mosquito breeding locations is one of the goals with respect to the educational efforts of several public health institutes in the counties involved in the present study. Education is conducted by means of lectures in schools, via pamphlets, which are distributed to all

households, and through the media. The mosquito control programme of the City of Zagreb requires the public health institute and certified companies to react promptly to complaints by citizens and to perform mosquito control.

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