

**STEGOMYIA ALBOPICTA SKUSE, 1894 (DIPTERA: CULICIDAE)
ON LUŠTICA PENINSULA 2011-2012 (MONTENEGRO)**

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Abstract - Luštica Peninsula in Montenegro was chosen as a study area to provide data about the distribution and population density of *Stegomyia albopicta*. This research is a preliminary step in the possible application of the sterile male technique (SIT). Fifty-four ovitraps were placed at 9 sites in August-September 2011-2012 to check peak seasonal activity. All of the nine sites were positive for *St. albopicta* in 2011; while in 2012, two of the nine places were negative. The most continuous presence of adults was registered at the locality of Tići, where they were collected throughout the entire sampling period. The highest number of eggs per trap (674) and in total was collected at Krašići in September 2011. At the localities Mrkovi and Begovići with a rare incidence of adults, the smallest number of eggs was counted. The two-year-long study showed that a well-established population of *St. albopicta* is persistently present on the peninsula, pointing to the possibility of testing the potential of SIT strategy in suppressing populations.

Key words: *Stegomyia albopicta*, *Aedes albopictus*, oviposition traps, Luštica peninsula, SIT, Montenegro

INTRODUCTION

Stegomyia albopicta [*Aedes albopictus*] (Skuse 1894) or Asian Tiger Mosquito (ATM) (Diptera, Culicidae) is considered one of the world's 100 worst invasive alien species (www.issg.org). The origin of the species is in South Eastern Asia, including Bangladesh, Cambodia, India, Indonesia, Laos, Malaysia, Nepal, Pakistan, Philippines, Singapore, Sri Lanka, Taiwan, Thailand, Vietnam, China, South Korea and Japan (Novak, 1992) (Fig. 1). In the region of origin, the species develops in small water bodies found in tree holes, leaf axils, coconut shells and other natural and artificial water containers.

St. albopicta is considered an opportunistic feeder; it strongly prefers mammals in general and

humans in particular. Aggressive biting activity is mainly concentrated during the daytime, unusual for native European mosquito species, with a peak late in the afternoon and in the morning during the hot summer months. Due to its high anthropophily and the ability to build and maintain large populations in urban and suburban areas, *St. albopicta* strongly affects the quality of life of millions of people in Europe and many more in the world. This species has the status of dangerous species in almost all infested countries because of its vectorial capacity to transmit several arboviruses such as Chikungunya (CHIK), dengue (DEN), St. Louis encephalitis (SLE), eastern equine encephalitis (EEEV), La Crosse encephalitis (LACV), Potosi (POT) and displays laboratory competence for 24 arboviruses and some microfilarial worms (Tiawsirisup et al., 2004;

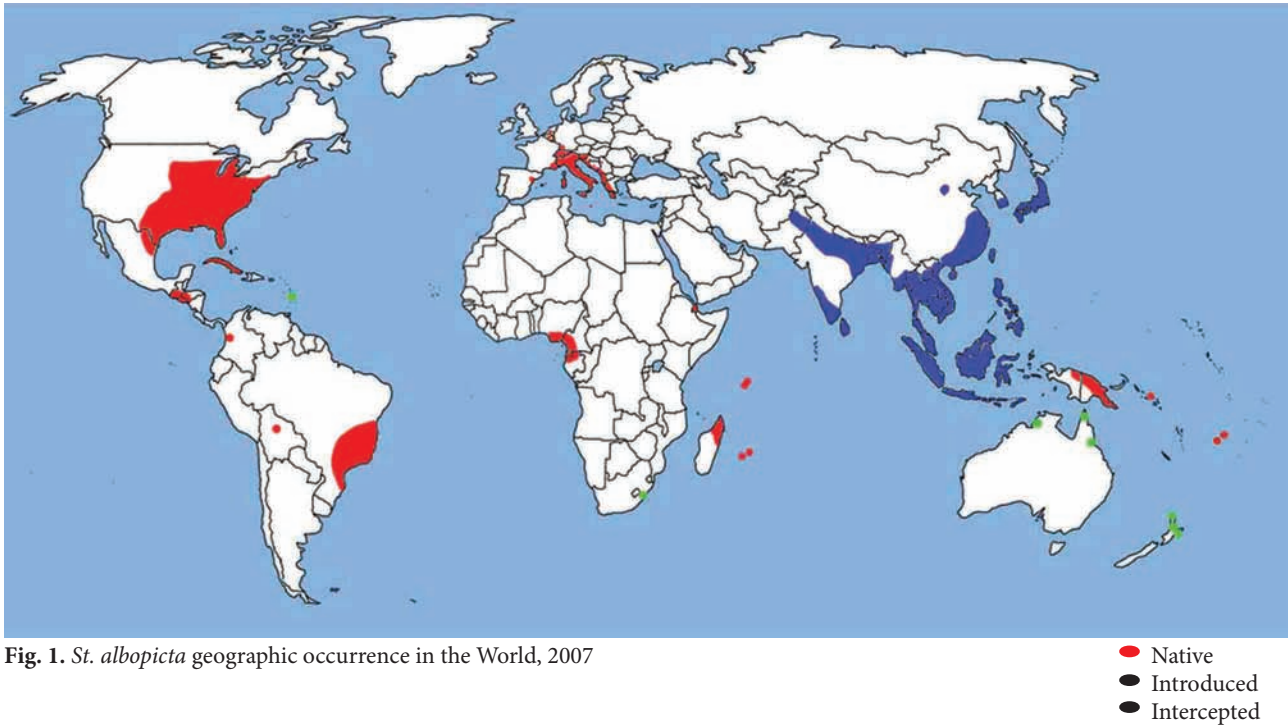


Fig. 1. *St. albopicta* geographic occurrence in the World, 2007

Honorio et al., 2003; Gerhardt et al., 2001; Gubler, 1998; Mitchell et al., 1992; Shroyer, 1986; Savage et al., 1994; Mitchell et al., 1996; Swanson et al., 2000; Novak, 1995; Debboun et al., 2005). In the Mediterranean basin, and rest of Europe where it is present, *St. albopicta* has the potential to become involved in the transmission cycles of Sindbis (SIN), West Nile (WN), Israel turkey meningoencephalitis (ITM), Tahyna (TAH), dengue (DEN), yellow fever (YF), African horse sickness (AHS), Rift Valley fever (RVF), Batai (BAT) and Japanese encephalitis (JE) viruses (Vazeille-Falcoz et al., 1999; Rebora et al., 1993; Cancrini et al., 2003a; Mitchell, 1995a and b; Cancrini et al., 2003b; Romi et al., 2004; Hribar et al., 2003). *St. albopicta* is an efficient vector of canine *Dirofilaria immitis* (dog heart worm) (Novak, 1995; Rodhain, 1995), and *Dirofilaria repens*, which are frequent parasites of dogs, other carnivores and humans, increasingly spreading into non-endemic areas of Europe (Otranto et al., 2013).

In the summer of 2007, *St. albopicta* was responsible for an epidemic of Chikungunya affecting some

250 people in the Emilia-Romagna region of Italy, close to the Adriatic coast (Angelini et al., 2007). It started spreading all over the world by invading Albania at the end of the 1970s. Nowadays is obvious that the spread of the ATM has been hastened by interstate shipments of used tires, as well as the movement of vehicles hosting adults on short and medium distances. In a new environment, the species is highly efficient in utilizing a range of artificial water containers (e.g., vases, dishes, tins, pots, flower pot plates, bottles, cemetery urns, urban water drainage systems and especially used tires) for the deposition of eggs. Used tires are usually stored outdoors, and after each rainfall an accumulation of water occurs inside the tires, being enough for *St. albopicta* to complete its life cycle (Reiter and Sprenger, 1987; Reiter, 1998; Lyon and Berry, 1991). Its global distribution is enhanced by the capability to overwinter in the egg-stage and therefore to permanently colonize temperate areas. A January isotherm of -3°C , July 20°C and annual rainfall of 500 mm in more than 60 rainy days delineate the areas of risk for *St. albopicta* establishment.

Up until 1962, scientific opinion was that this species is not capable of competing successfully with other related species. However, the species successfully and rapidly spread from the Indo-Malayan region to Japan (inhabiting bamboo stumps, discharged tires etc.) and outwards from Japan due to the worldwide trade of used tires. After its first detection outside its native distribution area in Albania (1979), in 1985 it became established in the USA, Texas. *St. albopicta* was recorded in 1983 in Memphis Tennessee, but established breeding populations were not observed before 1985 (Moore, 1986; Craven et al., 1988; Hawley et al., 1987; Snow and Ramsdale, 2002). Today in the USA the species is established in 25 states, including 678 counties (Moore and Mitchell, 1997) and it becomes a common member of the mosquito fauna in the southern states and states in the eastern half of the country (area bordered by line Minnesota-New Jersey, New Jersey-Florida, Florida-Texas, Texas-Minnesota) (Fig. 1).

In Central America, infested countries are Mexico from 1988 (Ibanez and Martinez, 1994; Rodriguez-Tovar and Ortega-Martinez, 1994), Dominican Republic (Pena et al., 2003), 1995 Guatemala (Ogata and Lopez-Samayoa, 1996) and Cuba from 1993 (Marquetti et al., 2000). Later on ATM established in Nicaragua (Lugo et al., 2005), Honduras, El Salvador, Barbados (Urbanelli et al., 1999), Panama and Costa Rica (Eritja et al., 2005). *St. albopicta* is found in South American countries, or parts of these countries, as well: in Brazil (Marques and Gomes, 1997) in 1986, then in Bolivia in 1995 (Eritja et al., 2005), Columbia in 1997 (Eritja et al., 2005), Argentina in 1998 (Rossi et al. 1999, loc. cit. in Vezzani and Carbajo, 2008) and Uruguay in 2003 (Rossi and Martinez, 2003 loc. cit. in Vezzani and Carbajo, 2008).

The species was also introduced into some countries of Africa: South Africa 1989 (Hunt et al. 1990, loc. cit. in Knudsen, 1995), Cameroon 1999 (Fontenille and Toto, 2001), Equatorial Guinea (2001) and Nigeria 1991 (CDC, 1991, loc. cit. in Knudsen, 1995) where it was implicated in an epidemic of yellow fever.

There are some data about the introduction of *St. albopicta* in New Zealand in 1994 (Calder and Laird, 1994, loc. cit. in Knudsen, 1995), in Australia (1988) in the Northern Territory and southern part (Johansen et al., 2005; Russell et al., 2005), and in the same year on the Fiji Islands (Laille et al., 1990, loc. cit. in Rodhain, 1995), but they were intercepted and promptly eliminated.

In Europe, the species was first reported in Albania in 1979 (Adhami and Murati, 1987; Adhami and Reiter, 1998). The next record is in 1990 in Italy, where it was introduced to Genoa by used tires imported from the USA (Sabatini et al., 1990; Romi, 1995; Dalla Pozza and Majore, 1992). After this, the species started its rapid spread across Europe. Firstly, it was found in northern France (Normandy) in 1999, also imported with used tires from the USA (Schaffner and Karsh, 1999). It was eradicated from the infested spots at that time, but today ATM is widely spread in the Mediterranean part of France, next to the Italian border. The species was also reported from Belgium in 2000 (Schaffner et al., 2004). In 2001, ATM was found in the state of Montenegro (Petrić et al., 2001), then in Switzerland, probably Hungary in 2003, but without confirmation to date (Scholte and Schaffner, 2007), and in Croatia and Spain in year 2004 (Klobučar et al., 2006; Aranda et al., 2006 loc. cit. in Kampen and Schaffner in Bonnefoy et al., 2008). The first record of the species in Croatia was in Zagreb in October 2004. In 2005, numerous colonies were registered at on the Istrian peninsula, Zadar, Split and Dubrovnik. It continued to spread rapidly, resulting in the colonization of the islands of Cres, Lošinj, Hvar and Vis. In 2005, the species was found in Bosnia and Herzegovina (Banja Luka), Greece, the Netherlands and coastal Slovenia (sea resort Portorož) (Petrić et al., 2006.; Samanidou-Vayadjoglou et al., 2005; Scholte et al., 2007). In 2007 it was intercepted in Germany (Björn et al., 2008) and 2009 established on Malta (Buhagiar, 2009).

The first specimen of *St. albopicta* in Montenegro was registered in Podgorica on August 21st 2001 (Petrić et al., 2001): there is no clear evidence of in-

roduction from neighboring Albania or from Italy. During the period 2002-2011 the species was found in Podgorica (Petrić et al., 2003), Bar, Ulcinj, Tivat, Andrijevica, Budva, Herceg Novi (Petrić et al., 2006), Kotor and around Skadar Lake (Pajović et al., 2012). From the year 2012, the project "Surveillance of invasive invertebrate *St. albopicta* in Montenegro" was launched, allowing a systematic approach aimed at depicting both the distribution and abundance of *St. albopicta* across Montenegro in the next three years. In this paper, the results of preliminary surveillance prior the start of the project are presented to provide the most suitable settlements for pilot ATM sterile male release in Montenegro.

MATERIALS AND METHODS

Luštica peninsula in Boka bay, Montenegro, was chosen for the study (Fig. 2). The peninsula is 13 km long, having a maximum width of 6 km and a total surface area of 47 km². The highest point of the peninsula is Obosnik peak at 582 m. It has a 35 km of coastline which accounts for 12% of the Montenegrin coastline. Luštica is a touristic semi-urban and rural area, divided between the Herceg Novi and Tivat municipalities. In Boka bay and Luštica peninsula, the average annual temperature is 14°C. In the coldest month, January, the temperature is above 0°C. During the summer, the average temperature is 25°C. Total precipitation in 2011 was over 1100 mm, with a typically Mediterranean regime with most precipitation during the winter and spring period. The vegetation belongs to the European-Mediterranean subregion (*Quercion ilicis*, Horvatić S., 1967), with a strong human influence, typified by degradation of natural vegetation of Mediterranean macchia, olive orchards and pinus monoculture (*Pinus halepensis*, *P. pinea* and *P. pinaster*).

The Luštica peninsula was chosen because the area is separated from the rest of the continental part of Montenegro and as such is very interesting. It would be interesting to import, release and test biological control agents or beneficial organisms as control measures against mosquitoes. Sterile male insects are included in the list of beneficial organisms.

However, for such an experiment two conditions are needed: a somewhat separate area (Luštica peninsula is ideal) and knowledge of the bionomics of the mosquito population in the area, as zero point. This was the main reason for the research whose results we are presenting in this paper.

In the survey program, we used plastic ovitraps (Schaffner et al., 2012a; Bellini et al., 1996). The choice was made based on the usefulness of plastic ovitraps in the field: unit cost, shock resistance, the possibility of making a hole so that, should it rain, water can drain and consequently maintain constant water levels, and they can be stacked during transport. The oviposition traps consisted a black plastic container (total height, 15 cm: height of water gauge, 10 cm: diameter, 11.5 cm) filled with rainwater and with a plywood strip (15x2.5 cm) inside as an oviposition substrate. The ovitraps were placed in green, shaded and easily accessible areas. They were positioned on the ground, with a free space above of at least 1 m (Carrieri et al., 2012). During the survey in 2011 and 2012, nine sites were monitored three times in the period of the year in which mosquitoes are the most active, from the end of August to the beginning of September. The trap stations were kept fixed, and the strips and water were collected and replaced at time intervals of 7-9 days, except for the first sampling interval in 2011 when the traps were checked after 14 days. All samples were labeled and brought to the laboratory in the Biotechnical Institute for identification. The number of eggs was counted by observing the oviposition strips under a microscope. All strips were dried before counting the eggs. After counting, they were placed in glass pots with the same collected water from the traps, for adult emergence. Mosquitoes were identified morphologically using diagnostic keys (Becker et al., 2003; Schaffner et al., 2012b). In parallel, we practiced visual observation and human bait catches (HBC) for adult detection.

RESULTS

In 2011, all nine sites were positive for *St. albopicta* adults and during the survey period in 2012, seven

Table 1. Adults detected visually or by human bait catches sampling

Site	Adults observation					
	2011			2012		
	20.08.	04.09.	11.09.	26.08.	03.09.	12.09.
Krtoli	-	+	+	+	-	+
Krašići	-	+	-	+	+	+
Bjelila	-	+	+	+	-	+
Franciškovići	-	-	+	-	+	-
Mrkovi	-	+	-	-	-	-
Tići	+	+	+	+	+	+
Zambelići	+	+	+	+	+	+
Begovići	-	+	-	-	-	-
Radovići	-	+	+	+	+	+



Fig. 2. Luštica peninsula and sites monitored in 2011-2012.

- sites monitored during 2011. (all positive)
- sites monitored during 2012. (positive)
- sites monitored during 2012. (negative)

out of nine places were positive (Fig. 2). The most constant adult population was recorded at the locality Tići where adults were observed during every

sampling visit (Table 1). At localities Zambelići and Radovići only once, at the beginning of survey, were we not able to confirm adult presence. The lowest

Table 2. The number of eggs in ovitraps sampled during the survey.

Site	Number of eggs / ovitrap											
	2011						2012					
	20.08.	04.09.	11.09.	Mean	SD	variability coefficient (%)	26.08.	03.09.	12.09.	Mean	SD	variability coefficient (%)
Krtoli	139	84	62	95	32.38	34.09	83	166	124	124.33	33.89	27.25
Krašići	79	674	59	270.67	285.32	105.41	412	81	250	247.67	135.14	54.56
Bjelila	331	205	18	184.67	184.67	69.63	29	33	189	83.67	74.50	89.04
Franciškovići	45	156	16	72.33	60.33	83.42	85	143	216	148	53.60	36.21
Mrkovi	45	43	8	32	16.99	53.09	61	89	38	62.67	20.85	33.28
Tići	62	102	84	82.67	16.36	19.79	58	25	3	28.67	22.60	78.84
Zambelići	34	244	68	115.33	92.03	79.80	122	67	104	97.67	22.90	23.44
Begovići	62	72	3	45.67	30.44	66.66	12	8	7	9	2.16	24.00
Radovići	268	132	112	170.67	69.31	40.61	247	296	369	304	50.13	16.49
Mean	118.33	190.22	47.78			61.39	123.22	100.89	144.44			42.57

infestation was observed at localities Mrkovi and Begovići, where only once (2011) in both years of the survey were we able to detect presence of adults.

Throughout the survey, 54 ovitrap samples were collected. All ovitraps were positive in both years. The total numbers of eggs sampled at different sites for both years are given in Table 2. During 2011, in 27 samples from nine sites a total 3.207 eggs were counted with the average number of eggs ranging from 47.78 eggs/trap in the third week of sampling to 190.22 eggs/trap in the second week. During 2012, the same number of traps at the same sites provided a total 3.317 eggs, the highest average number of 144.44 eggs/trap was registered in the third week, and the lowest number of 100.89 eggs/trap in the second week of sampling.

In both years, the highest average number of eggs per trap was recorded at Krašići (259.16 eggs/trap), with a maximum of 674 eggs/trap at the beginning of September 2011 and minimum of 59 eggs/trap in the middle of September of the same year. The average number (for both years) of more than 100 eggs/trap was also registered at Radovići (237.33 eggs/trap), Bjelila (134.16 eggs/trap), Franciškovići

(110.17 eggs/trap), Krtoli (109.67 eggs/trap) and Zambelići (106.50 eggs/trap). It is interesting that in Krašići with the highest average number of eggs/trap, adults were detected in only 4 out of 6 observation periods.

At Tići, where a continuous presence of ATM adults was observed, a low average number of eggs/trap was observed (55.67). The other two sites that were characterized by a high frequency of adult recordings, Zambelići and Radovići, yielded much higher two-year average egg counts of 106.5 and 237.33 eggs/trap, respectively.

At the localities Mrkovi and Begovići, the low occurrence of adults (Table 1) corresponded to the lowest numbers of eggs in both localities (47.33 and 27.33 eggs/trap, respectively).

DISCUSSION

The results of preliminary surveillance conducted prior to the implementation of the project "Surveillance of invasive invertebrate *St. albopicta* in Montenegro" are presented in this paper and discussed in order to establish the most suitable settlements

for a pilot ATM sterile male release in Montenegro. Villages of different size and degrees of isolation on the Luštica peninsula in Boka bay, Montenegro, were chosen to provide data about the presence and characteristics of ATM population in the area. As ATM larvae may occur in a wide-range of natural and artificial aquatic habitats, the monitoring of the ATM population by oviposition traps was implemented.

All sampling sites provided positive egg counts throughout the sampling periods in both 2011 and 2012. Moreover, in 2011 all sites were positive for *St. albopicta* adult presence and during 2012, ATM adults were detected at seven out of nine sampling sites. The most frequent occurrence of adults was at locality Tići, where they were detected during all the sampling periods despite the relatively low number of 55.67 eggs/trap. At other places (Mrkovi i Begovici) the least frequent adult counts were correlated to lowest numbers of eggs deposited in the traps (27.33 and 47.33 eggs/trap) The three highest average numbers of eggs per trap in both years were recorded at localities Krašići (259.16 eggs/trap), Radovići (237.33 eggs/trap) and Bjelila (134.16 eggs/trap), but were not clearly correlated to adult occurrence.

This pilot investigation has shown that *St. albopicta* is widely spread on the Luštica peninsula, with quite a stabile variation in local population sizes. This provides a good foundation for the planning of SIT application. The next step will be to extend the monitoring period in order to cover the entire seasonal development dynamic in order to determine the start of the first generation. This is important for planning the most appropriate SIT strategy.

Acknowledgments - This research was supported through the project "Surveillance of invasive invertebrate *St. albopicta* in Montenegro" financed by Ministry of Science of the Republic of Montenegro (2012-15), and in part by grants III 43007 and TR 31084 of Ministry of Education and Science, Republic of Serbia.

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