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ORIGINAL ARTICLE

First Record of *Stegomyia albopicta* in Turkey Determined By Active Ovitrap Surveillance and DNA Barcoding

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Abstract

Despite its confirmed establishment in neighboring Greece and Bulgaria, the presence of the Oriental invasive species *Stegomyia albopicta* (Skuse) (=*Aedes albopictus*) has never been confirmed in Turkey. Active surveillance for this container-breeding species was carried out using oviposition traps at 15 discrete sites in the towns of Ipsala ($n=8$ sites), Kesan ($n=5$) (Edirne District), and Malkara ($n=2$) (Tekirdag District) in the Thrace region of northwestern Turkey, from May 23 through November 10, 2011. Eggs collected were reared to the fourth larval instar and adult stages where possible to facilitate integrated morphological and molecular species identification. DNA barcodes (658 bp of the mitochondrial cytochrome *c* oxidase I [*COI*] gene) were compared with all four potentially invasive *Stegomyia* species: *St. aegypti*, *St. albopicta*, *St. cretina*, and *St. japonica.* Sequences generated for samples collected in Thrace Region were herein confirmed as *St. albopicta*, the first record of this vector species in Turkey. Eggs of *St. albopicta* were detected in two discrete localities: (1) In the grounds of a restaurant in Kesan (in week 36), and (2) in the customs area of the Turkish–Greek border at Ipsala (in weeks 32 and 38). Multiple detection of *St. albopicta* eggs indicates the possible establishment of the species in northwestern Turkey. Finding this important disease vector has implications for public health and requires the implementation of active vector monitoring programs and targeted vector suppression strategies to limit the spread of this invasive vector species in Turkey.

Key Words: *Stegomyia albopicta*—*Aedes albopictus*—Turkey—Establishment—DNA barcoding.

Introduction

Stegomyia albopicta (Skuse) (Diptera: Culicidae) (=*Aedes [Stegomyia] albopictus*; Reinert et al. 2004), commonly known as the Asian tiger mosquito, is an important vector of some 22 arboviruses in the families Flaviviridae (*e.g.*, dengue, West Nile, yellow fever, Japanese encephalitis), Bunyaviridae (*e.g.*, Rift Valley fever, Potosi, Cache Valley, and LaCrosse viruses), Togaviridae (*e.g.*, chikungunya and Ross River virus) (Rosen 1986, Mitchell 1995a, Mitchell et al. 1998, Gratz 2004, Tilston et al. 2008). After *St. aegypti, St. albopicta* is the secondary epidemic vector of dengue and dengue hemorrhagic fever (WHO 1999). In Europe, the species acts as a dengue vector and was most recently incriminated as the only vector in the first European outbreak of chikungunya in northeastern Italy (Rezza et al. 2007).

Under laboratory conditions, *St. albopicta* is shown to be able to function as a bridge vector for West Nile virus (WNV) between the enzootic *Culex* spp.–avian cycle and susceptible mammalian hosts, including humans (Turell et al. 2001, Sardelis et al. 2002). *St. albopicta* is also implicated in zoonotic disease cycles, indicating that the species also feeds on other mammalian hosts. *St. albopicta* is a competent vector of the filarial nematodes *Dirofilaria immitis* and *D. repens* and is a natural vector of *D. immitis* in Italy (Cancrini et al. 2003a,b). Because of its aggressive anthropophilic behavior, *St. albopicta* could also enhance the zoonotic circulation of filarial nematodes from animals to humans in an urban environment (Cancrini et al. 2003a).

The global invasion of *St. albopicta,* originally native to subtropical southeastern Asia, islands of the Western Pacific and Indian Ocean, into the Pacific islands, Africa, the Caribbean, the Middle East, the Americas, and Europe, has been well documented (*e.g.*, Gratz 2004, Benedict et al. 2007). The species was first detected in Europe from Albania in 1979 (Adhami and Murati 1987), and its establishment has been

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reported in 12 further European countries and microstates to date: Croatia, France, Greece, Monaco, Montenegro, Italy, Malta, San Marino, Slovenia, Spain, Switzerland, and Vatican City (Benedict et al. 2007, Scholte et al. 2007, ECDC 2009, Gatt et al. 2009). The presence of *St. albopicta* in Belgium, Bosnia and Herzegovina, Germany, and The Netherlands has been documented, but these reports are believed to represent accidental importations, rather than established populations at present (Scholte et al. 2007, ECDC 2009, Gatt et al. 2009). In addition, the presence of *St. albopicta* in Bulgaria was reported at a scientific meeting in 2011, but as yet this remains unpublished (Ognyan Mikov, pers. comm.); the species has been recently identified in Bolshoi Sochi Region of Russia (Anonymous 2012).

Distribution models predict that *St. albopicta* will continue to expand depending on transport, environmental, and climatic changes (Knudsen et al. 1996, Medlock et al. 2006, Benedict et al. 2007). The majority of *St. albopicta* introductions worldwide are reportedly due to dormant egg transfer via the international trade in used tires (Reiter and Sprenger 1987) and shipments of the Asian plant "lucky bamboo" (*Dracaena* spp.) (Linthicum et al. 2003, Scholte et al. 2007) or by the inadvertent transport of adult mosquitoes in aircraft and other modes of transport (Gratz et al. 2000). Between 1989 and 2010, Turkey imported 1627 tons of used tires from 20 countries, including United States, Greece, Japan, South Korea, China, Taiwan, Thailand, and Singapore, where *St. albopicta* is known to be established and/or endemic (Unlu and Farajollahi 2012).

Turkey has been identified as one of several countries (Cyprus, Bulgaria, Macedonia, Portugal, and southern Russia) under high risk of invasion due to its favorable climatic conditions and geographical proximity to established populations of *St. albopicta* (Mitchell 1995b, Knudsen et al. 1996, Benedict et al. 2007, ECDC 2009, Fischer et al. 2011, Caminade et al. 2012, Unlu and Farajollahi 2012). The suitability for the establishment of *St. albopicta* in Turkey has been confirmed by risk maps using different analytical techniques (*e.g.*, Random Forest model, Multi-Criteria Decision Analysis, 0 and −5°C isotherm analysis, mean yearly temperatures, annual rainfall) and shows highest suitability in the coastal parts and lower likelihood of establishment in mountainous regions (ECDC 2009, Caglar and Karacaoglu 2011, Unlu and Farajollahi 2012).

Turkey has 18 major ports and 11 border gates, which are mostly located in areas with favorable climatic conditions for *St. aegypti* establishment. The Thrace region embraces both the Kapikule (Turkey–Bulgaria) and Ipsala (Turkey–Greece) border gates, which are the main trade routes between Turkey and the rest of Europe. This part of Turkey was considered at especially high risk, because *St. albopicta* is already established in northern regions of neighboring Greece (Corfu, Igoumenitsa, and Serres) (Samanidou-Voyadjoglou et al. 2005, Unlu and Farajollahi 2012). Following the advice of the European Centre for Disease Prevention and Control (ECDC) (2009), an active surveillance program was established in the Thrace Region of northwestern Turkey near to the Greek border in the summer of 2011, with the explicit aim of determining whether this invasive vector species was present and/or established in Turkey.

Materials and Methods

Study area

Fifteen sites in three districts (Ipsala, Kesan, and Malkara) were selected for active surveillance in the Thrace Region of northwestern Turkey, based on the climatic suitability of the region for *St. albopicta* establishment as stated by previously determined risk maps (ECDC 2009, Unlu and Farajollahi 2012) (Table 1, Fig. 1).

Ipsala province (106 km^2; population 8033) is located 108 km southwest of Edirne city and 2 km east of the Greek border. The customs gate in this area represents the most intensive transport route between Greece and Turkey for import/export of goods, and facilitates high numbers of person transits between the nations, including tourists. The north and eastern parts of Ipsala are surrounded by small hills (100–300 meters), whereas the Ipsala plain, with its intensive rice cultivation, dominates the western area. The natural borders of Ipsala correspond to the Meric River in the west and the Ergene River in the northwest. Continental climate prevails in Ipsala, with considerable seasonal and daily fluctuations in temperature. Intensive wet rice cultivation increases the relative humidity in summer (Ipsala Government 2012).

Kesan province (1087 km^2; population 54,367) is 112 km south of Edirne, some 30 km east of Ipsala province and 26 km west of Malkara province, respectively. The province is dominated by lowland plains (~100 meters above sea level [a.s.l]), but the Korudag mountains in the southeast rise to 371 meters a.s.l.. The Marmara type of Mediterranean climate prevails in Kesan, which is more moderate at the southern coastal areas on the Aegean Sea (Saros Bay). The mean annual precipitation is 48.8 mm with 75.6% relative humidity, and the mean annual temperature is 13.7°C in Edirne. During the mosquito season, from May to September, the mean relative humidity is 72% and mean temperature is 21.8°C (min 11.4°C and max 31.7°C) (Kesan Government 2012).

Malkara Province (1225 km^2; population 28,788) lies 56 km west of Tekirdag and shares a similar geography to Kesan, composed mainly of lowland plains. The Tekir Mountains, 25 km south of Malkara, have their maximum altitude at Ganos temple (845 meters a.s.l.). A semicontinental climate prevails in Malkara, which is a transition type between the Mediterranean and Black Sea climates. The mean annual precipitation is 48.3 mm with 85% relative humidity. During

Table 1. Coordinates and Altitude of Sampling Stations and Number of Ovitraps Used per Site

Stations[a]	Coordinates		Altitude (meters)	Ovitraps/site
	N	E		
K1	40.87556	26.67306	88	3
K2	40.88694	26.65306	46	3
K3	40.85611	26.63917	100	3
K4	40.87667	26.64139	43	3
K5	40.89250	26.64694	65	3
I1	40.89222	26.42417	60	3
I2	40.93944	26.38222	11	3
I3	40.94250	26.38806	12	3
I4	40.90250	26.38611	18	3
I5	40.92639	26.35917	10	5
I6	40.92389	26.31750	10	8
I7	40.93111	26.34722	7	5
I8	40.92111	26.37667	15	3
M1	40.89389	26.91028	206	3
M2	40.90694	26.92000	210	3

[a]K, Kesan District; I, Ipsala District; M, Malkara District.

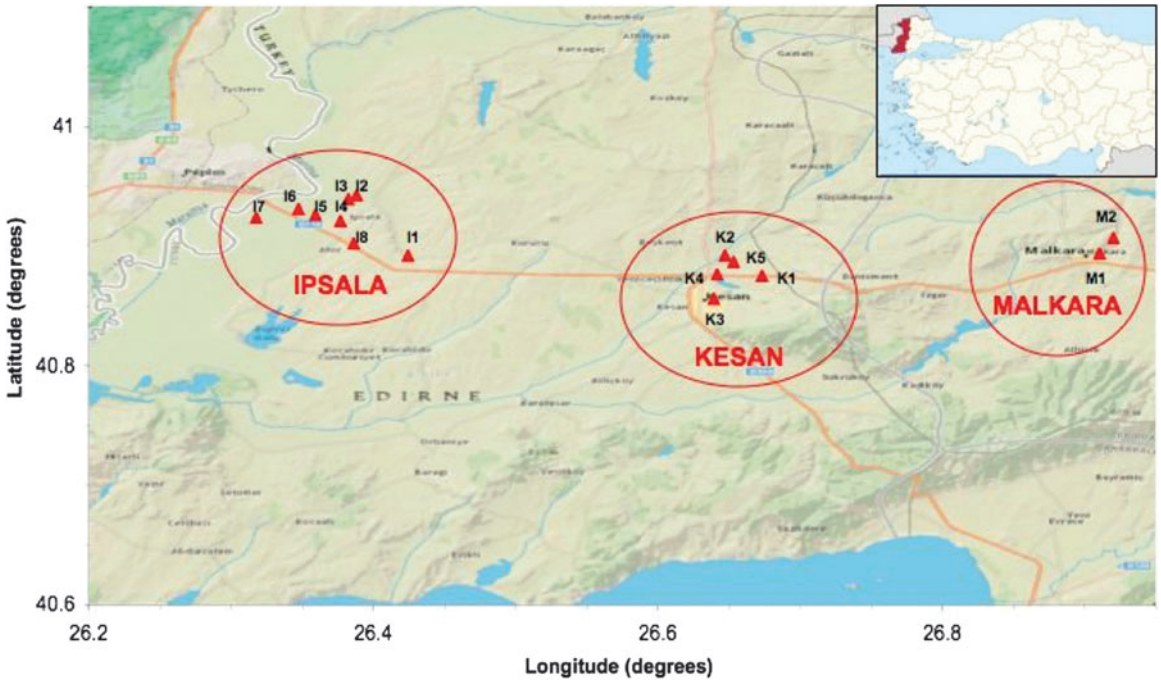

FIG. 1. Map detailing the localities of sampling stations in the Thrace Region of northwestern Turkey. I: Ipsala, (1) I1: Truck parking area, (2) I2: Buket Street, (3) I3: Cikmaz Street, (4) I4: Building gardens, (5) I5: Government fish breeding farm, (6) I6: Ipsala customs area, (7) I7: Rice cultivation company, (8) I8: Tire repair shop. K: Kesan, (9) K1: Truck parking area, (10) K2: Supermarket parking area, (11) K3: University campus, (12) K4: Park, (13) K5: Restaurant gardens. M: Malkara, (14) M1: Cami Street, (15) M2: Exhibition Center gardens (see Table 1 for site coordinates).

the mosquito season, from May to September, the mean relative humidity is 79% and mean temperature is 21°C (minimum 12.4°C, maximum 27.9°C) (Malkara Government 2012).

Surveillance program

Surveillance activities were conducted between May 23 and November 10 (weeks 22–42) of 2011. Guided by the risk analysis reports (ECDC 2009, Unlu and Farajollahi 2012), we considered the main possibility of entry to be the passive transportation of adults/eggs from Greece via the main trade motorway (E84). Therefore sampling stations were determined at urban and periurban areas along the E84 motorway (Fig. 1). Some 54 ovitraps were set up at 15 locations (Ipsala, $n=8$; Kesan, $n=5$; Malkara, $n=2$). Ovitraps were positioned on the ground in green, shady, and easily accessible areas, with a free space of at least 1 meter above, and checked at biweekly intervals (Albieri et al. 2010, Carrieri et al. 2011). Exact altitude and coordinates of sampling stations, and numbers of ovitraps set per site are given in Table 1. Location coordinates and altitudes were estimated using a Magellan® Explorist 5100® GPS receiver.

Ovitraps comprised black plastic cylindrical containers (diameter 11.2 cm, height 11.8 cm, volume 1.1 L). Each contained a single Masonite-like oviposition strip (150 × 23 mm) and 600 mL of water containing *Bacillus thuringiensis israelensis* Vectobac® 12AS formulation (stock = 1200 International Toxic Units [ITU]/mg) at a dose of 1 mL/L (equivalent to 1279 ITU/mL) to avoid possible larval development according to the protocol of Carrieri et al. (2009). Ovitraps were checked biweekly; oviposition strips were transferred into labeled, airtight plastic bags and transported to the laboratory in a temperature-insulated cooler box without ice. Oviposition strips were individually examined for mosquito eggs using a stereomicroscope in the laboratory.

To facilitate morphological identification, collected eggs collected were reared through to final instar larvae and adults following the published protocol of Medici et al. (2011). Eggs were collected from the oviposition strips using soft brushes, transferred onto filter paper, and submerged into labeled containers filled with dechlorinated water. A total of 4 mg of crushed dry cat food (Friskies®) was provided per larva for the whole larval development, with 10% given on day 0, 45% on day 2, and the rest on day 5. Specimens that died as immatures were stored individually in Eppendorf® tubes containing 96% ethanol at −20°C prior to molecular analysis. Adults were preserved dry at −20°C for later morphological species confirmation.

Morphological identification

Morphological identification of adult male and female mosquitoes was carried out using the available keys of Schaffner et al. (2001) and Becker et al. (2003). Egg characters were compared to those highlighted in Linley (1989).

Molecular identification

DNA was extracted on the QIAgen® BioSprint robotic platform using the BioSprint 96 DNA Tissue Kit (QIAgen®, Crawley, England, UK), following the manufacturer's instructions. The barcode region of the mtDNA cytochrome *c* oxidase I (*COI*) gene (658 bp, less primers) was amplified using the universal LCO and HCO barcoding primers (Folmer

et al. 1994) using the standard Mosquito Barcoding Initiative (MBI) protocols, expressly listed in Ruiz et al. (2010). PCR products were purified using the Millipore® vacuum manifold system, following the manufacturer's instructions. Bidirectional DNA sequences were generated in the Sequencing Facility of the Natural History Museum, London, using the Big Dye® Terminator Kit (PE Applied BioSystems®, Warrington, England) and run on an ABI 3730 automated sequencer (PE Applied BioSystems®).

Sequences were edited using Sequencher™ version 4.8 (Genes Codes Corporation, Ann Arbor, MI) and compared with published sequences in GenBank and other unpublished sequences in the Mosquito Barcoding Initiative Dataset in the Barcode of Life Database (BOLD, www.boldsystems.org/). Sequences used for comparison in this study were based on those *Stegomyia* species previously reported in Europe, including *St. albopicta* (JQ004524-25 and HQ398900-01) and *St. japonica* (HQ978777-78), and three specimens of *St. cretina* from Greece and four specimens of *St. aegypti* from Kenya from the MBI dataset, now available in GenBank as accessions KC250445–KC250447, and KC250441–KC250444, respectively. Sequence statistics, calculation of pairwise distance parameters using Kimura's two-parameter algorithm (Kimura 1980), and the construction of the bootstrapped neighbor-joining trees (Felsenstein 1985, Saitou and Nei 1987) were carried out in MEGA v. 5.0 (Tamura et al. 2011).

Voucher DNA extracts are stored in the long-term frozen collection of the Natural History Museum, London (BMNH), where they can be accessed on request. Bidirectional edited *COI* electropherograms and specimen details of the three Turkish *St. albopicta* specimens sequenced (including exact localities with georeferences and specimen identifiers) are freely available in the Mosquito Barcoding Initiative project STHAW (*Stegomyia* of Turkey) in the BOLD database (Ratnasingham and Hebert 2007), and appear in GenBank as barcode red flag data, indicating their high quality and voucher standards under the accession numbers JQ412504–JQ412506. Morphological vouchers, as larvae and adults of both sexes, are held in the laboratory of the senior author (K.O.) and are available for examination on request.

Results

Ovitrap surveillance carried out in 15 sampling stations in the Thrace Region of Turkey from May to October 2011 yielded 4872 mosquito eggs (Table 2). More than 90% of all the eggs collected ($n = 4828$) were those of *Culex pipiens* sensu lato (s.l.), and the remaining 44 eggs were identified as those of the genus *Stegomyia.* Although the ovitrap surveillance began in May, 2011, the first *Stegomyia* eggs were detected in Ipsala (site I6; $n = 20$ eggs) in August, 2011 (week 32) (Table 2, Fig. 2b,c).

DNA barcoding was employed to verify the identity of four small larvae preserved in ethanol from site I6 in Ipsala. DNA fragments corresponding to 658 bp of the mitochondrial *COI* region were amplified and sequenced from three of these specimens and compared with DNA from specimens of all four species of *Stegomyia* previously detected in Europe: *St. aegypti*, *St. albopicta*, *St. cretina*, and *St. japonica*, publicly available in GenBank, or generated through the efforts of the MBI (Fig. 3). The common *COI* haplotype from these individuals was100% identical with *St. albopicta* GenBank entry JQ004525 (Fig. 3), thus confirming the presence of *St. albopicta* in Turkey for the first time.

The *COI* sequences of all three specimens of *St. albopicta* sequenced from ovitrap I6 were identical, indicating that these individuals may well represent siblings from the same mother (*i.e.*, one egg batch) (Table 3). Intraspecific variation was low (< 0.1%) in *St. albopicta* ($n = 7$), *St. aegypti* ($n = 4$), and *St. cretina* ($n = 3$), and slightly higher (0.8%) in *St. japonica* ($n = 2$). Genetically, *St. albopicta* is most closely related to *St. cretina* (11.5% sequence difference), than *St. aegypti* (13.8%) and *St. japonica* (14.5%) (Table 3, Fig. 3)

Table 2. Numbers of Eggs of *Stegomyia albopicta* (Indicated by Gray Shading) and *Culex pipiens* Collected in the Biweekly Ovitraps by Sampling Station

		Week																						
		22		**24**		**26**		**28**		**30**		**32**		**34**		**36**		**38**		**40**		**42**		
Station	**No. of ovitrap**	*C.p*	*S.a*	*C.p*	*S.a*	*C.p*	*S.a*	*C.p*	*S.a*	*C.p*	*S.a*	*C.p*	*S.a*	*C.p*	*S.a*	*C.p*	*S.a*	*C.p*	*S.a*	*C.p*	*S.a*	*C.p*	*S.a*	**Total**
K1	**3**	0	0	0	0	0	0	35	0	123	0	59	0	41	0	32	0	21	0	0	0	0	0	**311**
K2	**3**	0	0	0	0	0	0	0	0	0	0	24	0	22	0	0	0	0	0	0	0	0	0	**46**
K3	**3**	0	0	0	0	0	0	28	0	25	0	0	0	20	0	43	0	20	0	0	0	0	0	**136**
K4	**3**	0	0	0	0	0	0	45	0	40	0	0	0	35	0	30	0	38	0	0	0	0	0	**188**
K5	**3**	0	0	0	0	25	0	34	0	93	0	125	0	335	0	204	**18**	67	0	37	0	30	0	**968**
I1	**3**	0	0	0	0	0	0	0	0	30	0	35	0	20	0	42	0	29	0	0	0	0	0	**156**
I2	**3**	0	0	0	0	0	0	34	0	42	0	48	0	0	0	61	0	32	0	0	0	0	0	**217**
I3	**3**	0	0	0	0	14	0	23	0	0	0	32	0	61	0	56	0	32	0	0	0	0	0	**218**
I4	**3**	0	0	0	0	16	0	31	0	56	0	85	0	105	0	64	0	0	0	35	0	22	0	**414**
I5	**5**	0	0	0	0	10	0	19	0	27	0	31	0	0	0	45	0	26	0	0	0	0	0	**158**
I6	**8**	0	0	0	0	38	0	63	0	106	0	114	**20**	98	0	73	0	66	**6**	54	0	0	0	**638**
I7	**5**	0	0	0	0	0	0	13	0	37	0	24	0	36	0	0	0	28	0	0	0	0	0	**138**
I8	**3**	0	0	8	0	30	0	0	0	221	0	97	0	154	0	124	0	60	0	22	0	27	0	**743**
M1	**3**	0	0	0	0	0	0	45	0	0	0	101	0	62	0	86	0	75	0	0	0	0	0	**369**
M2	**3**	0	0	0	0	0	0	33	0	43	0	0	0	20	0	46	0	30	0	0	0	0	0	**172**
Total	**54**	0	**0**	8	**0**	133	**0**	403	**0**	843	**0**	775	**20**	1009	**0**	906	**18**	524	**6**	148	**0**	79	**0**	**4872**

FIG. 2. Photographs of the two ovitrap locations where *St. albopicta* eggs were detected in this study: (**a**) Site K5 in Kesan District, and (**b** and **c**) site I6 in Ipsala District. In **a**, the white arrow indicates the position of the ovitrap on the fence.

Subsequent and closer inspection of fourth instar larvae and the 12 adult specimens reared from the ovitrap at site I6 also confirmed the presence of *St. albopicta* by morphology and served to provide pristine voucher specimens of this alien taxa. All *Stegomyia* eggs detected in this study were reared to optimize correct morphological verification.

Eggs of *St. albopicta* were obtained in two discrete localities in Kesan (ovitrap K5) and Ipsala (ovitrap I6) districts of the Thrace Region of western Turkey, some 30–60 km from the Turkish-Greek border (Fig. 1, Tables 1 and 2). In Ipsala, 20 *St. albopicta* eggs were collected from an ovitrap set in shrubbery near to the Ipsala border gate (I6: Figs. 1 and 2b,c) in week 32 and a further 6 eggs collected from the same ovitrap in week 38 (Table 2). One other positive *St. albopicta* collection ($n = 18$ eggs) was from an ovitrap set in the grounds of a restaurant in Kesan (site K5; Figs. 1 and 2a), 30 km east of Ipsala City, in week 36 (Table 2). At sites K5 and I6, *St. albopicta* eggs were collected in sympatry with egg rafts of *Cx. pipiens* s.l. That *St. albopicta* eggs were collected in two localities in the Thrace Region of Turkey, some 30 km distant from each other, and at the same site in Ipsala 6 weeks apart, suggests that *St. albopicta* is not only present in northwestern Turkey, but has in fact established low-level populations in this area.

In a concurrent survey, three adult females *Stegomyia* were detected a gravid trap in vegetation in Ipsala City, on July 12, 2011. Although these specimens were tentatively identified as *St. cretina* in the field, we are now more inclined to believe that these specimens were also *St. albopicta*. No other *St. cretina* specimens were detected in this study.

In this study, eggs of *Cx. pipiens* s.l. were collected from almost all sampling stations ($n = 4828$ in 67 egg rafts) (Table 2). During first week of trapping (week 23), no egg rafts were collected, and in the subsequent 2 weeks, collections were occasional. However from weeks 26 to 38, collections became consistent and more intense. Among the sampling stations, K5 in Kesan and I8 and I6 in Ipsala yielded the most *Cx. pipiens* s.l. eggs, with totals of 968, 743, and 638 eggs, respectively. The population size of *Cx. pipiens* s.l. showed unimodal increase. During the study period, the population of these mosquito species was found to be lowest in June ($n = 141$). Population size increased during July ($n = 1379$) and August ($n = 1784$), with the highest peak in August (week 34), and decreased during September ($n = 1430$) and October ($n = 227$).

Discussion

Herein, the presence of *St. albopicta* was confirmed for the first time in western Turkey in August, 2011, through retrospective correlation of 658-bp mitochondrial DNA *COI* gene sequences ("DNA barcodes") with those of morphologically verified specimens sequenced by the MBI. DNA barcoding was employed to facilitate identification from early larval instars (1–3), which cannot reliably be identified using taxonomy alone. Given that potentially invasive taxa are rarely

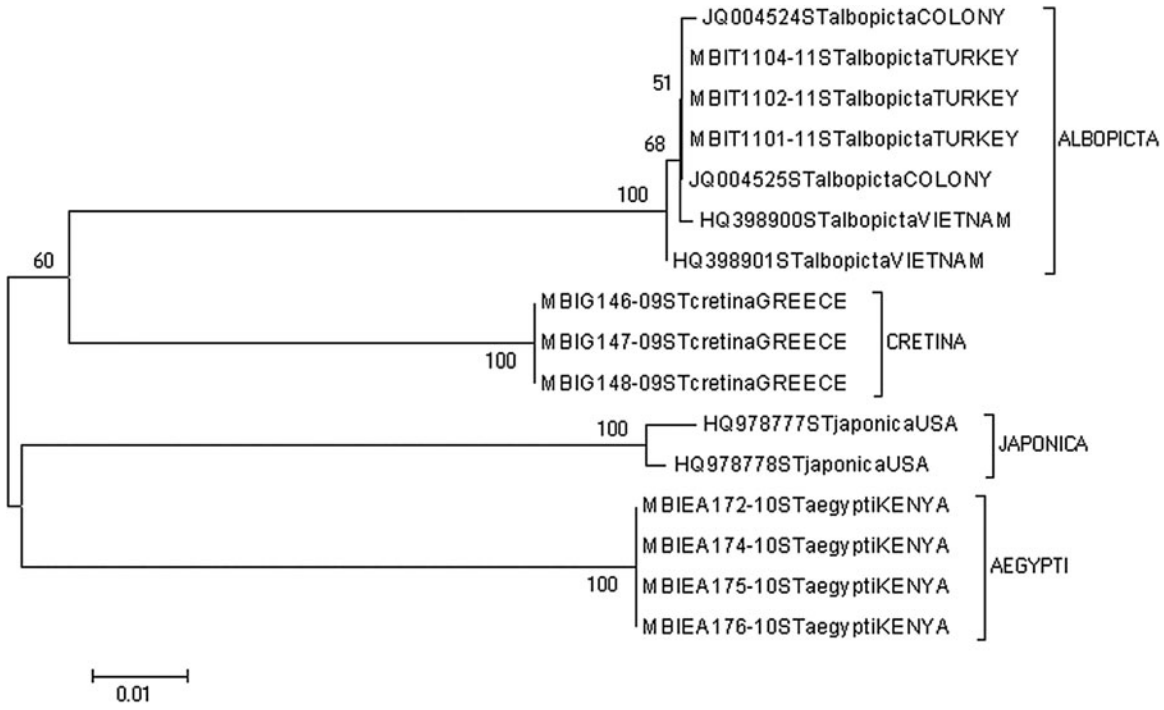

FIG. 3. Bootstrap neighbor-joining tree constructed using 1000 replicates of Kimura two-parameter distance model genetic distance matrices (Kimura 1980) of cytochrome *c* oxidase sequences (658 bp) belonging to 16 specimens of the four *Stegomyia* species previously reported in Europe; *Stegomyia albopicta* ($n=7$; 3 from Turkey herein, HQ398900-1, JQ004524-5), *St. aegypti* ($n=4$, herein), *St. cretina* ($n=3$, herein), and *St. japonica* ($n=2$; HQ978777-8). The optimal tree with the sum of branch length = 0.26529964 is shown. The percentages of replicate trees in which the associated taxa clustered together in the bootstrap test are shown next to the branches (Felsenstein 1985). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. Evolutionary analyses were conducted in MEGA v.5.0 (Tamura et al. 2011).

included in most commonly used regional keys, we advocate the molecular confirmation of newly encountered invasive species to conclusively confirm the identity of alien taxa (Scholte et al. 2007 and herein). Once the identity of an alien taxa has been confirmed, morphological identifications can be employed for routine screening.

The prediction that "the climatic suitability of Turkey would dictate that it is only a matter of time before the species is introduced and established" (Unlu and Farajollahi 2012) and an earlier ECDC report (2009) directly led to the implementation of this active surveillance study in western Turkey and clearly shows the value of predictive risk modeling of potentially invasive vector species in previously identified localities (Mitchell 1995b, Knudsen et al. 1996, Benedict et al. 2007, ECDC 2009, Fischer et al. 2011, Caminade et al 2012, Unlu and Farajollahi 2012). Along with Turkey, Bulgaria and southern Russia were highlighted to be at high risk of invasion, and the presence of *St. albopicta* in these zones was reported at a scientific meeting in 2011 (see Introduction) and in early 2012 (Anonymous 2012), respectively. Given that these predictions were valid in Bulgaria, other countries highlighted in these previous studies (Cyprus, Macedonia, Portugal, and southern Russia) should take these warnings seriously and undertake active surveillance programs (ECDC 2009, Unlu and Farajollahi 2012). This study supports the effectiveness of biweekly ovitraps for the detection of *St. albopicta,* as employed in Italy (Albieri et al. 2010, Carrieri et al. 2011), and we herein support this methodology as a simple cheap and effective method for active surveillance of this invasive species. That *Cx. pipiens* s.l. eggs were collected in the ovitraps indicate that this method can also be used to effectively monitor *Cx. pipiens* s.l. populations, perhaps in a combined virus vector surveillance program in future.

That *St. albopicta* is reported in western Turkey herein for the first time is of significance to public health authorities in Turkey. The ability of *St. albopicta* to use both natural and artificial containers for larval habitats facilitates its widespread occupation of urban and periurban environments

Table 3. Mean Inter- and Intraspecific DNA Sequence Divergence Using 658 bp of the mtDNA *COI* Genes of *St. albopicta* (ALBO, $n=7$), *St. cretina* (CRET, $n=3$), *St. japonica* (JAP, $n=2$), and *St. aegypti* (AEG, $n=4$)

	AEG ($n=4$)	CRET ($n=3$)	JAP ($n=2$)	ALBO ($n=7$)
AEGYPTI	**0.000**			
CRETINA	0.131	**0.000**		
JAPONICA	0.136	0.119	**0.008**	
ALBOPICTA	0.138	0.115	0.145	**0.001**

Genetic distances were generated in MEGA v. 5.0 (Tamura et al. 2011) using Kimura's two-parameter algorithm (Kimura 1980). Intraspecific distances are indicated in boldface.

(Hawley 1988), ensuring a close connection between the species and the human population, increasing the risk of vector-borne diseases in these areas (Takken et al. 2007). The spatial and temporal confirmation of the species in two different locations of the Thrace Region, 30–60 km from the Greek–Turkish border in this study, indicates that not only is the species present in the country, but also that indeed the species may well be established in this region of Turkey, albeit at low population density at the current time.

St. aegypti was reported in the Aegean port city of Izmir and nearby Odemis in Turkey in 1961, but this was believed to be an accidental importation and the species did not establish in this region (Curtin 1967). *St. cretina*, native to Greece, is the only species of the genus native to Europe, yet invasions of *St. albopicta*, *St. aegypti*, and *St. japonica* have all been reported (Curtin 1967, Adhami and Murati 1987, Benedict et al. 2007, Scholte et al. 2007, Gatt et al. 2009, ECDC 2009, Schaffner et al. 2009, Anonymous 2012), and the latter three predicted in Turkey (ECDC 2009, Unlu and Farajollahi 2012). Herein we show that DNA barcodes readily separate *St. albopicta*, *St. aegypti*, *St. cretina*, and *St. japonica* and this technique should prove useful in determining the true identity of invasive *Stegomyia* species detected across Europe in future.

Geographic information systems (GIS) models recently presented by Caglar and Karacaoglu (2011) showed the possible expansion pattern of the species in Turkey, indicating that most regions of the country were suitable for occupation by *St. albopicta*; thus, efforts must be undertaken to prevent the establishment of the species. Early detection of *St. albopicta* through active surveillance coupled with targeted suppression measures to prevent establishment is promoted as the most cost-effective method of *St. albopicta* control (Mack et al. 2000, Derraik 2006, ECDC 2009). Conventional larval control strategies (*e.g.*, insecticide spraying, larval site management) have proven highly ineffective in controlling already established, high-density *St. albopicta* populations due to the dispersal and availability of larval sites (Mack et al. 2000, Derraik 2006). Due to typical discontinuous distribution and low active dispersal potential and ease of mass-rearing (Bellini et al. 2007), one of the most promising control measures to date is the sterile insect technique (SIT), which requires sustained effort and funding.

Governmental-level policy to avoid the invasion of etiological agents and the insects that carry them is generally underdeveloped in Europe, reflecting the low level of threat to which we have previously been exposed. Island countries, including Australia and New Zealand, are extremely tight on border controls to avoid accidental importation of insect disease vectors and agricultural pests, and these countries maintain well-established early warning systems including active surveillance. Implementation of a program to prevent the establishment of *St. albopicta* and associated viruses/parasites transmitted by the species in Turkey was suggested by Ergunay et al. (2011).

Given the detection of *St. albopicta* in Turkey's western Thrace Region herein, we advocate that active surveillance must now be implemented not only in northwestern Turkey, but also in other regions of the country that are at risk. *St. albopicta* has been reported in Latakia, Syria, which is only 60 km south from the Yayladagı border gate of Hatay, Turkey (Haddad et al. 2007). The Turkish–Syrian border has 12 border gates along the frontier, with the Yayladagı border gate facilitating one of the major immigration and trade routes through the Hatay province. Turkey is also at risk from passive introduction of mosquitoes in vehicles coming from neighboring countries with established populations of *St. albopicta* (Bulgaria, Greece and Syria), onboard boats coming from Greece and Italy, and as desiccated eggs in used tire importations.

Although border zones are key to the introduction of invasive species, the role of internal transportation (of both the mosquitoes and the diseases they transmit) via human movement should not be overlooked. Internal movements of large numbers of agricultural workers and laborers between South Eastern Anatolia and the Cukurova plain and areas in the Mediterranean, Aegean, and Thracean provinces could result in these persons returning to the central plateau and eastern highlands, carrying the vector-borne pathogens and some of the vectors with as well (Alten et al. 2007, Takken et al. 2007).

Our confirmation of *St. albopicta* in Turkey coupled with the recent detection of dengue seroactivity in humans in Ankara (Ergunay et al. 2010) compounds the need for active detection and elimination practices of this species in Turkey. Further investigations will be conducted during the 2012 season to determine the extent of this incursion in Turkey, survey additional areas and actively initiate control measures.

Conclusions

This study documents the presence of *St. albopicta* in Turkey for the first time in August, 2011, and supports the strategy of active surveillance programs in areas identified as high risk through geographical, climatic, and ecological niche modeling. DNA barcoding has proven an accurate diagnostic method of confirming the identity of early instar *St. albopicta* and differentiating it from other morphologically similar invasive species in Europe (*St. aegypti*, *St. cretina*, and *St. japonica*), thus we advocate this method for the rapid confirmation of alien taxa in future active ovitrap surveillance surveys.

That *St. albopicta* has been detected in low density in western Turkey implies that targeted vector control strategies can still be employed in the region to avoid the establishment of high-density populations of this effective arboviral vector. Given the serious diseases *St. albopicta* is known to transmit to humans, prevention of the establishment of this species should be regarded as a high priority for public health authorities in Turkey.

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Author Disclosure Statement

No competing financial interests exist for any of the authors involved in this manuscript.

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