

First Record of *Aedes (Stegomyia) albopictus* in the Netherlands

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Abstract

In the summer of 2005, *Aedes (Stegomyia) albopictus* (*Stegomyia albopicta* in Reinert *et al.*, 2004) was found inside and around three different horticultural companies near Aalsmeer in the Netherlands. The species was identified both by morphology and ITS2-DNA sequencing. Although a few adults were also found outdoors, it remains unclear whether these were “escapes” or if indeed the species is reproducing outdoors in or near the site of introduction. This record constitutes the northernmost finding of *Ae. albopictus* in Europe to date at 52°N. The introduction is associated with the import of *Dracaena* plants, colloquially known as ‘lucky bamboo’, from China.

Keywords: *Aedes albopictus*, *Dracaena sanderana*, lucky bamboo, Dengue, the Netherlands

Introduction

Aedes (Stegomyia) albopictus (Skuse, 1894) (Diptera: Culicidae) (*Stegomyia albopictus* in Reinert *et al.*, 2004) is commonly referred to as the ‘Asian tiger mosquito’. This species is considered a serious threat to human health because of its potential to transmit at least 22 types of arboviruses (Mitchell, 1995a; Gratz, 2004) and other infectious agents including *Dirofilaria immitis* (Cancrini *et al.*, 2003; Comiskey & Wesson, 1995). It is especially notorious for its vectorial capacity of all four serotypes of dengue (Mitchell, 1995a), which is transmitted both horizontally and vertically (Guo *et al.*, 2004). *Aedes albopictus* was also circumstantially incriminated as the principal vector of Chikungunya virus in la Reunion and other islands in the Indian Ocean, where a major epidemic of this arbovirus occurred in 2005 (Paganin *et al.*, 2006).

Aedes albopictus was originally indigenous to South-East Asia, including parts of China, Korea, Japan, and islands of the Western Pacific and Indian Ocean. Range expansion eastwards across Pacific islands during the early 20th century was mainly due to the increased trade in used tyres, and, in recent decades, it has spread to Africa, the Middle East, the Americas (north and south), and Europe (Knudsen, 1995; Mitchell, 1995b). In Europe, the species has been reported from Albania (Adhami & Reiter, 1998), Italy (Sabatini *et al.*, 1990), France (Schaffner & Karch, 2000; Schaffner *et al.*, 2001), Spain (Roiz *et al.*, 2006), Belgium (Schaffner *et al.*,

2004), Switzerland (Flacio *et al.*, 2006) Greece (Samanidou-Voyadjoglou *et al.*, 2005), Serbia and Montenegro (Petric *et al.*, 2006), Croatia (Petric *et al.*, 2006), Bosnia and Herzegovina (Petric *et al.*, 2006), and Slovenia (Petric *et al.*, 2006; Schaffner, 2006).

In July 2005, a routine inspection of a horticultural company (henceforth called Company 1) in the municipality of Haarlemmermeer was carried out by the Dutch National Plant Protection Service (NPPO). Company 1 had recently imported *Dracaena sanderana*, also known as ‘lucky bamboo’ or ‘Ribbon Plants’ from China. The inspector reported the presence of large numbers of an unknown mosquito species on the premises of the company where the *Dracaena* plants were kept: a relatively small hall with doors that were left completely open wide to the outside. The inspector was bitten by several mosquitoes and described them as ‘aggressive’. No larvae were found. The personnel of Company 1 and of a neighbouring (non-horticulture) company in the same building mentioned that for several weeks they had experienced severe mosquito nuisance and mosquito bites during daytime working hours. This study was carried out in order to identify these mosquitoes.

Materials & Methods

Following the initial inspection, specimens were captured, tentatively identified as *Ae. albopictus* and sent to the Natural History Museum, London for morphological and molecular verification. Molecular

identification was carried out by PCR amplification and sequencing of the nuclear ITS2 sequences using the 5.8SF and 28SR primers of Collins & Paskewitz (1996) following the protocol listed in Linton *et al.* (2001).

Results

All specimens tested were identified as *Ae. albopictus*. Sequences obtained were 100% identical to those entered for *Aedes albopictus* entries AY741376 and M95127, and varied only by a single base to AY741377.

Subsequent visits of mosquito specialists of the Dutch NPPO and of the Laboratory of Entomology of Wageningen University to the company to place traps and visually search for mosquitoes reconfirmed the presence of *Ae. albopictus*, but in contrast to the inspector's reported massive abundance of mosquitoes in Company 1, only few (<5) specimens were found. One month after the confirmation of *Ae. albopictus* in Company 1, this company moved to another location. Despite continued monitoring at the original site of the findings, no other findings of *Ae. albopictus* were reported from company 1 until February 2006, when 2 adult mosquitoes were caught outdoors, some 15 meters from the original site of Company 1.

A few weeks after this first report, similar mosquito nuisance was reported from two other *Dracaena*-importing companies, both in the neighbouring municipality of Aalsmeer. Companies 2 and 3 kept the *Dracaena*-plants in large greenhouses under controlled climatic conditions. The doors that connect the greenhouses with the outdoor environment were usually kept closed, except during on/offloading of trucks. At the initial survey of Company 2 and Company 3, few *Ae. albopictus* were collected, despite complaints of mosquito nuisance, but shortly afterwards several larvae and adults of *Ae. albopictus* were found in the greenhouse of Company 2. Once the presence of *Ae. albopictus* had been confirmed, this company applied chemical insecticides (type unknown) in an attempt to control and contain the mosquitoes. In Company 3, several adults were found, and one larva. On two occasions at the end of September, two adults were collected outdoors of Company 3, some 20 meters from the entrance. Although Company 3 had sprayed chemical insecticides (larvicides? Or against adults?) upon experiencing mosquito nuisance, mosquitoes continued to be collected from Autumn 2005 through to the spring of 2006.

Sampling was carried out approximately once every fortnight by visual inspection and by using 'counterflow' traps ('Liberty Plus' – American Biophysics Corp., North Kingstown, RI 02852, USA). The traps were used as a continuous monitoring device and were placed shortly after the first finding of *Ae. albopictus*. Until November 2005 traps were only placed outdoors, one at each company, 10-15 m from the main entrance to the hangar and greenhouse, respectively. From November 2005, an additional trap was placed indoors at each company. The traps were emptied and checked for the presence of *Ae. albopictus* approximately once every fortnight. The results are presented in Table 1.

Discussion

Aedes albopictus is mainly introduced into new areas via the international trade in used tyres (Knudsen, 1995). However, the introduction of *Ae. albopictus* into the Netherlands has been shown to be connected to the import of *Dracaena sanderana* plants, as was reported in California in 2001 (Linthicum *et al.*, 2003; Madon *et al.*, 2004). Both confirmed cases in California were linked to sea-trailer import of these plants from southern China. *Dracaena sanderana* is a popular ornamental plant in Europe. The plants are transported in Perspex boxes containing a few centimetres of water. We propose that the mosquitoes reported from the Netherlands derived from eggs that had been deposited near the water level, either on the plants or the Perspex boxes, while the plants were still in southern China.

Since the region of southern China from which the *Dracaena* plants originated is endemic for dengue (CDC, 2005), this introduction of *Ae. albopictus* in the Netherlands could constitute health risks, both to the employees in the infested greenhouses, and to inhabitants of the surrounding areas. Since dengue can be transmitted transovarially in *Ae. albopictus* (Zhang & Zhang, 1996), the risk of dengue infection to humans in the Netherlands derives not only from mosquitoes that arrived from China already as adult mosquitoes, but also for newborn generations. It is likely that *Ae. albopictus* will be able to survive in greenhouses, but it is unclear whether it is able to reproduce in the natural climatic conditions of the Netherlands and whether it could establish itself. The distribution of *Ae. albopictus* in the north of Asia occasionally reaches the -5°C isotherm. Even assuming a conservative 0°C isotherm, this means that the species might become established in northern

Date	Company 1				Company 2				Company 3									
	Indoors	Outdoors			Indoors	Outdoors			Indoors	Outdoors								
	<i>Ae. albopictus</i>	<i>Ae. albopictus</i>	<i>Cx. pipiens</i>	<i>Cs. annulata</i>	<i>Ae. albopictus</i>	<i>Cx. pipiens</i>	<i>Ae. albopictus</i>	<i>Cx. pipiens</i>	<i>Cs. annulata</i>	<i>An. maculipennis</i>	<i>Ae. albopictus</i>	<i>Cx. pipiens</i>	<i>Cs. annulata</i>	<i>Ae. albopictus</i>	<i>Cx. pipiens</i>	<i>Cs. Annulata</i>	<i>Cx. modestus</i>	<i>Aedes sp.</i>
Adults collected by mosquito net																		
July 2005	<5				<5						<5							
Larvae collected in July 2005																		
					2						2							
Adults collected in Counterflow CQ traps																		
26-08-05											1							
02-09-05							8		1				1	57		4		
23-09-05			3	3			9						2	45	1			
03-10-05											2							
07-10-05			15	1			8							30				
13-10-05			3				3							8				
03-11-05				1			9				1			28	2			
11-11-05			1	1			5							8				1
02-12-05			55	1			4				11	2		10				
09-12-05			14								1	1	1					
22-12-05			8								9	1						
06-01-06											3	3						
16-01-06											6	1						
27-01-06			1								2		1					
09-02-06		2	2								1							
23-02-06			1								1							
03-03-06			1															
15-03-06												1						
30-03-06						2					1							
18-04-06			1			5	1	9				1					1	
02-05-06			3			1	12	6	2	1							2	
16-05-06			2			6	20	1			2						1	

Table 1: *Aedes albopictus* and other Culicidae collected indoors and in the environs of three horticultural companies in the Netherlands.

Europe as far as the southern coast of Sweden and Norway, including the Netherlands (Mitchell, 1995b). Kobayashi *et al.* (2002) write that the northern limits of *Ae. albopictus* in Japan is between latitudes 38-40° N. Using Geographical Information System (GIS) software, they proposed that *Ae. albopictus* could survive in global regions where the annual mean temperature is higher than 11°C and the mean temperature of the coldest month is not lower than -2°C. Furthermore, the period with temperatures above 11°C should successively continue for more than 186 days per year.

This record of *Ae. albopictus* in the Netherlands is the northernmost finding in Europe so far, at between approximately 52.15-18° N, which is considerably further north than previous records in Japan (Kobayashi *et al.*, 2002) and in the USA (Moore & Mitchell, 1997). The annual mean temperature in the Netherlands is coldest at 8.9-9.2°C in the northeast, to 10.1-10.4°C in the southwest. The lowest mean temperature in the coldest month of the year in the coldest area (northeast) of the Netherlands is 2°C (KNMI, 2002). These climatic data suggest that it is unlikely that *Ae. albopictus* could establish itself in the Netherlands. However, its presence should be continuously monitored

and research should be conducted to verify this. The strain of *Ae. albopictus* from northern Japan is known to hibernate; egg diapause is induced at the onset of short day length. At present it is unknown if the *Ae. albopictus* strain from southern China can hibernate, and if so, what proportion of eggs may survive the Dutch winter. Research should be carried out to determine whether this strain could therefore survive and establish itself in the Netherlands. That immature stages of *Ae. albopictus* were recently found in Normandy (France) and Vlaanderen (Belgium) (Schaffner *et al.*, 2004), suggests that at least that strain was able to reproduce in climatic conditions comparable to those in the Netherlands. These latter introductions were associated with imported tyres and in both cases the species did not become established possibly because of the unfavourable winter conditions. However, in the current situation in the Netherlands there is a real possibility for establishment of *Ae. albopictus*, as the artificial landscape of greenhouses is very large and provides excellent environmental conditions for this species (constant and suitable climate, breeding sites, human blood hosts). During the summer, when outdoor conditions are suitable for *Ae. albopictus*, the mosquitoes can disperse into adjacent greenhouses and thus enlarge their distribution.

The introduction of *Ae. albopictus* into the Netherlands of this invasive mosquito species must be controlled, both because of its vector competence for all 4 dengue viral strains and other medically important viruses, and its nuisance caused by excessive biting. The import of *Dracaena* plants on water may facilitate the continuous import of this invasive species, and therefore pose health risks to those working at, or residing near to, these companies and greenhouses. Care should also be taken with plants exported from the Netherlands to other European destinations. Transit on gel rather than water is probably an effective way to reduce the presence of *Ae. albopictus* in 'lucky bamboo' imports. Other means of preventing further imports of these mosquitoes could include the mandatory treatment of shipping containers with larval biocides shortly before shipment. The capacity of *Ae. albopictus* to transmit a variety of pathogens of human and veterinary public health importance makes continued surveillance an important issue.

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