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First record of *Aedes (Stegomyia) albopictus* (Skuse) (Diptera: Culicidae) in Malta

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Abstract

Aedes albopictus (Skuse, 1894) is newly added to the mosquito fauna of Malta, which now numbers ten species. Its biology and distribution are briefly described and its establishment and possible modes of introduction to the island are discussed. The need for further field work and surveillance is highlighted.

Keywords

Mosquito, *Aedes albopictus,* Malta, introduction.

Introduction

The Maltese archipelago lying in the central Mediterranean consists of the inhabited islands of Malta, Gozo and Comino and a number of uninhabited islets and rocks, occupying an area of about 316 km^2. The islands, which lie 93 km south of Sicily and 288 km east of Tunisia, are semi-arid with a Mediterranean climate that is characterised by a mild, relatively short wet season, and a protracted, dry season. There are no mountains, and the highest point is 253m above sea level (Dingli, Malta). The average annual rainfall is 530 mm but this is highly variable. There are no lakes, rivers and streams, only minor temporary springs, and few, somewhat small, bodies of permanent freshwater exist. Air temperatures are moderate with a mean monthly temperature range of 12-26°C although short cold winter snaps with a minimum night time temperature of 1-3°C can occur between December to February. Relative humidity is consistently high throughout the year, being mostly in the range 65-80%. The islands are windswept and only about 8% of the days of the year are calm. The prevailing wind is the northwesterly Mistral which blows on about 19% of windy days

(Schembri, 1993; Cassar *et al*., 2008). Another significant wind is the northeasterly Gregale. Both winds can be strong, causing significant damage. The daylight hours vary from about 11.8 hours in July to 10 hours in December (Cutajar *et al.*, 1992).

Nine species of mosquito, all in the subfamily Culicinae, are currently reliably known from the Maltese islands (Ramos *et al*., 1992; Gatt, 1996, 2009). *Aedes albopictus* (*Stegomyia albopicta* of Reinert & Harbach, 2005), popularly known as the 'Asian tiger mosquito' originates in the forests of Southeast Asia, where it breeds preferentially in rotting tree holes. First described by Skuse (1894) from India, it has found its way throughout the world due to its ability to breed successfully in a wide range of both natural and artificial habitats that bear still bodies of water, including so-called artificial 'container habitats'. These include tyres, barrels, drinking troughs, overflow dishes for potted plants, vases and other receptacles which have accumulated rain water. That it lays cold and drought-resistant eggs and does not exhibit a marked host preference (Hawley, 1988) are other factors that have aided its successful dispersal. The transport of eggs with cargo which previously had water in it is the chief mode of transmission from country to country. The global trade in used tyres which have previously been stored outdoors has been mostly responsible (Hawley *et al.*, 1987; Knudsen, 1995; Reiter, 1998). Another significant mode of spread has been with the importation of eggs and larvae within the canes of 'Lucky Bamboo' (*Dracaena* spp.) (Madon *et al.*, 2003; Scholte *et al.*, 2007). Once the species is established in a new country, domestic traffic along roads and highways continues to spread it further, as the species is known to travel via vehicular movement across states. Transportation of adult mosquitoes by traffic from Italy to Switzerland was reported by Flacio *et al.* (2004). Similarly, Pluskota *et al.* (2008) demonstrated that adult mosquitoes can be transported over large distances by cargo trucks and tourists returning from Italy to Germany.

On the European mainland it was first discovered in Albania in 1979 (Adhami & Reiter, 1998), and later in the port of Genoa in Italy, where it was introduced in a shipment of used tyres from the United States (Sabatini *et al.*, 1990; Dalla Pozza & Majori, 1992). By 1994, Dalla Pozza *et al.* had reported it as already widespread in Italy (Dalla Pozza *et. al.,* 1994). Since that time it has been reported from Sardinia (Romi, 1995), France (Schaffner & Karch, 2000), Montenegro (Petrić *et al.*, 2001), Belgium (Schaffner *et al.*, 2004), Switzerland (Flacio *et al.*, 2004), Greece (Samanidou *et al.*, 2005), Croatia (Klobučar *et al.*, 2006), Spain (Aranda *et al.*, 2006), Bosnia and Herzegovina (Petrić *et al.*, 2006), Slovenia (Petrić *et al.*, 2006), the Netherlands (Scholte *et al.*, 2007) and Germany (Pluskota *et al.*, 2008). It is also known from the provinces of Palermo and Messina in Sicily (ECDC, 2009). *Aedes albopictus* has been listed as amongst the 100 most invasive species by the Invasive Species Specialist Group (ISSG, 2009), and Scholte & Schaffner (2007) and Enserink (2008) detail its rapid invasion into new territories.

The biology of *Ae. albopictus* has been extensively reviewed by Hawley (1988). It can best be described as a

semidomestic container breeder, and the most typical habitats are man-made containers and tree holes. Almost all mountainous areas higher than 500m above sea level are free of it (ECDC, 2009). Distinct cold-tolerant and tropical strains have been described (Knudsen, 1995). Its biology is diverse and depends on geographical and climatic factors.

Adult females are aggressive daytime biters and prefer to bite outdoors. They feed on a wide variety of hosts including humans, domestic and wild animals, reptiles and birds, but prefer mammalian blood. Drought-resistant eggs are laid singly in soil or above the water level in a container. Repeated inundations are required for the eggs to hatch. Larval development takes 3-8 weeks, and the species is multivoltine, resulting in 5-17 generations per year depending on rainfall and temperature. Adults live for up to three weeks, and are on the wing between April/May to November in temperate Europe. Their flight range is limited to less than 1 km (Reiter & Sprenger, 1987) and most disperse less than 180m during their lifetime (Bonnet & Worcester, 1946) so adults are never found far away from the larval habitats.

In tropical and subtropical regions, populations are active throughout the year, with no overwintering stage. In temperate regions, however, the species overwinters in the egg stage through egg diapause. Shortening daylight hours in autumn stimulate females to lay eggs that enter facultative diapause (Estrada-Franco & Craig, 1995). Hatching in spring is directed by environmental variability (climate and photoperiod). The first overwintering eggs hatch when daylight reaches 11 to 11.5 hours per day and the mean temperature is 10 to 11°C (Toma *et al.*, 2003). In less temperate regions, the species overwinters in the adult stage. Adult females have been reported to overwinter in Rome and ovitraps contained eggs in winter, indirectly proving that adult mosquitoes can survive throughout winter in suitable environments (Romi *et al*., 2006).

Aedes albopictus is an aggressive biter with a high nuisance value. More significantly, it is of great medical and veterinary importance as it is a known competent vector of at least 22 arboviruses (compiled in Gratz, 2004). The most important of these are dengue, yellow fever, chikungunya, Japanese encephalitis and West Nile viruses. It also transmits dirofilariasis to dogs. An outbreak of chikungunya fever in Taranto, Italy, where around 250 people were infected has been linked to this species (Angelini *et al.,* 2007). This outbreak is a cause of grave concern, and today 12 countries in Europe have implemented specific surveillance for this and other exotic mosquito species (ECDC, 2009).

For this reason, it is not only of interest but also of public health importance to report this species for the first time from Malta. The material examined was identified using the keys of Samanidou & Harbach (2001) and the interactive CD of Schaffner *et al.* (2001).

Material and location

MALTA: Limits of Ghajn Zejtuna, Mellieha: 13.ix.2009: 2 ♀♀, 1800h, in garden; 14.ix.2009: 2 ♀♀, in cistern; 15.ix.2009: 1 ♀, 0930h, biting man in garden; 15.ix.2009: 1 ♂, ex. pupa in cistern; 1 larva in cistern, pupated 3.x.2009, emerged 7.x.2009; 16.ix.2009: 2 ♀♀, in bedroom; 2 ♂♂ in cistern;

17.ix.2009: 2 ♀♀, 1900h, attempting to bite man in garden; 18.ix.2009: 4 ♂♂ and 4 ♀♀, in cistern; 19.ix.2009: 7 ♂♂ and 10 ♀♀, in cistern; 20.ix.2009: 7 ♂♂ and 7 ♀♀, in cistern.

Attempts to trap further material using light traps baited with dry ice between 18.00h and 24.00h on the 18th of September 2009 were unsuccessful.

All material was collected by two of the authors (PG, JCD) from an area in the limits of Ghajn Zejtuna, Mellieha, Malta (Fig. 1, No. 1) and is in part preserved in a private collection (PG) and in part in the collections of the National Museum of Wales (JCD).

The area in which the species was discovered is situated about 200m from the shoreline, which forms part of the north-eastern coast, and is largely exposed to strong winds. The site is surrounded by overgrown gardens and some derelict land on which dense thickets of semi-natural vegetation are present. It has a small, poorly closed cistern located in a shaded area, which collects rain water from surrounding flat roofs during winter. The water in the cistern appears to be clean and clear, with very little organic matter, but contains a bottom sediment derived from decaying leaves. Very few immature stages were present in the water, and repeated sampling resulted only in one larva and one pupa being collected.

A small valley overgrown with *Arundo donax*, Wied Ghajn Zejtuna, is situated about 500m to the west. The valley used to sustain a small permanent spring, which at the time of survey has appeared to have dried up, but still retains some water during the wet season (pers. obs., PG). Following the discovery of this species, control measures were applied to the water in the cistern and the surrounding gardens. The cistern was then pumped dry and properly sealed.

Discussion

Aedes albopictus is present in large parts of Italy, which is, by far, the most heavily infested country in Europe. Reports also indicate that it is present in many coastal areas of Sicily (ECDC, 2009). Considering the close proximity of the islands of Malta to Italy (and Sicily), and the large amount of sea traffic that occurs between Italy/Sicily and Malta, it is not surprising that the species has now been found in Malta. However, the time of this introduction, its origin and the risk of establishment of this species in Malta is still questionable.

The mosquitoes of the Maltese islands are still incompletely known, and only one additional paper has been published (Gatt, 2009) since the group was reviewed thirteen years ago (Gatt, 1996). The distribution of the 9 known species is also poorly known, and the larval habitats of some are still unknown. It would appear, however, that the species has only recently been introduced for three reasons. Firstly, field work carried out in Malta and Gozo by a mission from the European Centre for Disease Prevention and Control between the 6th of April and the 1st of May 2009, (at a period of time when the species shows activity in Italy) in which two of the authors (FS & PG) actively participated, and specifically targeted to detect the presence of exotic species failed to discover this species (Schaffner, 2009). This survey investigated selected sites where *Ae. albopictus* would most likely

be expected to occur, and involved sampling 513 larval habitat units including 91 man-made containers, light trapping with dry ice (20 night/traps, and placement of some ovitraps. Secondly, a BG-Sentinel trap run by one of the authors (PG) for one week at night in April 2009 in the area in which the species was now found failed to collect it, but some nights were rainy and windy. Thirdly, the locality was regularly visited by one of the authors (PG) over the past year, but the species was never observed. Even though these investigations failed to discover the species, the risk of introduction was still considered high, due to regular ferry traffic from continental Italy and Sicily (Schaffner, 2009).

The suitability of territories of Europe for *Ae. albopictus* has been recently evaluated using two models (ECDC, 2009). A statistical random forest model based on the presence/absence of data and retaining four variables related to temperature predict the extent of the species along the European Mediterranean coasts, as almost all of these areas appear as suitable, even if the southern Sicilian coast and Malta show a lower suitability level than the coasts of continental Italy. A second model (Multi Criteria Decision Analysis) based on expert advice, considers climate factors that should limit the establishment of the species, i.e. annual rainfall, summer and winter temperature. Also in that model output, Malta appears to be favourable for the species, and climate values fulfil the criteria laid down by the experts. Apart from the total annual rainfall levels, the distribution of rainfall throughout the season could limit the development of the mosquito, mainly in inland rural arid areas. But as the main larval habitats are man-made containers which are artificially flooded, the species should be able to proliferate in Maltese urban areas, where these containers are known to be abundant. Finally, climate data and abundance of suitable larval habitats may allow the survival of the species over the winter, as well as its establishment and proliferation. Overwintering should occur through diapausing eggs, but adults may also remain alive, as some females show laying activity throughout the winter in Rome (Romi *et al*., 2006).

Although the data so far are very limited, it is interesting to speculate about possible ways this species might have been introduced. Very little trade, if any, in used tyres occurs in Malta. Likewise insignificant numbers of Lucky Bamboo are imported by the garden centres close to Mellieha (Fig. 1, No. 2), and these are imported directly from the Netherlands. Although *Ae. albopictus* has been introduced several times to plant nurseries in the Netherlands, it has not become established there (ECDC, 2009). It is therefore very unlikely that these two potential sources of introduction were involved.

On the other hand, a large volume of sea traffic occurs between Italy/Sicily, and Malta. In 2002, 824 vessels carrying cargo or in ballast from Italy/Sicily (9.5% of the total number of foreign registered vessels) entered Maltese waters, mostly at the Malta Freeport in Marsaxlokk (Fig. 1, No. 3) (National Statistics Office, 2004). Italy/Sicily and Malta are also important holiday/trade destinations for both Italian and Maltese nationals, and although statistics are not available, a large number of both private and commercial vehicles cross over to

Malta from Italy/Sicily by ferry, docking in the Valletta harbour area (Fig. 1, No. 4). In the high tourist season, there are two ferry crossings carrying vehicles per week from Italy (Genoa, Civitavecchia) and up to 18 ferry crossings per week from Sicily (Catania, Palermo, Pozzallo). In the absence of evidence that the species is established around the Valletta ferry harbour, it is possible that it was introduced directly to Mellieha by travellers with vehicles returning from Italy/Sicily.

Another viable possibility is that the species was carried to the Mellieha coast from shipping by high winds blowing from the northwest. A shipping route is located offshore some 5 Nm from the coast (Fig. 1, No. 5). Likewise, in rough weather, ships can often be observed at anchor awaiting orders, some 1 Nm off shore at il-Prajjiet (Fig. 1, No 6) sometimes for days.

Urgent fieldwork is now necessary in order to determine whether *Ae. albopictus* is more widely distributed in Mellieha and neighbouring areas, and to apply control measures if it is found that it is, in order to try and prevent its spread and establishment. It will also be necessary through the use of ovitraps and BG-Sentinel traps to monitor the area where the mosquito was found to determine whether the species has become established there, if it will survive the winter, and if so, whether it will overwinter in the egg or adult stage. A winter isotherm of between -3° and 0°C is a limiting factor for establishment (Nawrocki & Hawley, 1987), a condition which is fulfilled by Maltese winters. The establishment of a stable population will depend on the initial size of the founder population (Hanski, 1999). Founder populations, even if small, may be reinforced by additional introductions which prevent them from dying out.

Also of paramount importance is the application of an efficient surveillance programme concentrating on ports (especially ferry harbours) utilising ovitraps and BG-Sentinel traps in order to monitor the presence of the mosquito at these potential introduction sites. If *Ae. albopictus* becomes established in Malta, its introduction to the sister island of Gozo will rapidly follow, as there is much traffic by ferry between the two islands. In 2008, some 20,000 trips were made between the islands, carrying more than a million private and commercial (cargo) vehicles (National Statistics Office, 2009).

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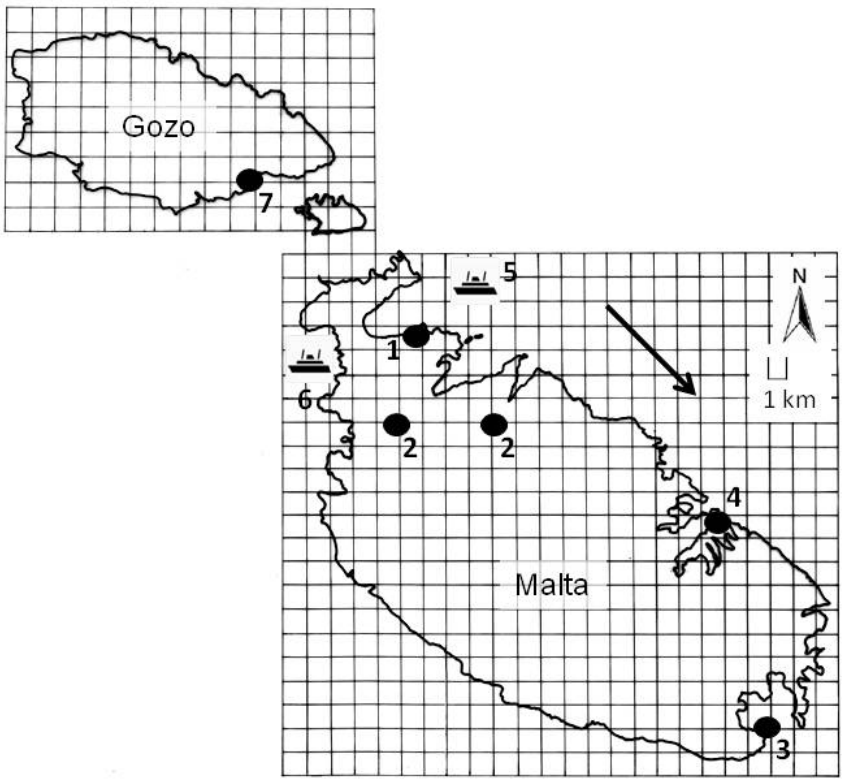

Figure 1. Map of Malta and Gozo showing: **1.** Limits of Ghajn Zejtuna, first observation of *Ae. albopictus*; **2.** Closest garden centres; **3.** Malta Freeport, Marsaxlokk; **4.** Valletta harbour; **5.** Offshore shipping routes; **6.** Ships at anchor, Il-Prajjiet; and **7.** Mgarr harbour. Arrow = Northwesterly wind