First record of *Aedes albopictus* (Skuse, 1894) (Diptera; Culicidae) from three islands in the Tyrrhenian Sea (Italy)

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Abstract: *Aedes* (*Stegomyia*) *albopictus*, an invasive mosquito originating from the Asian-Pacific region, is known as an important vector of several pathogens of public and veterinary health significance. In 1990, this mosquito was recorded for the first time in Italy (Port of Genova) and in about 20 years it has become established throughout the whole country. In this paper, we report for the first time the presence of *Ae. albopictus* on three small islands in the Tyrrhenian Sea, namely Giglio, Ventotene and Ustica. Most probably, the maritime transport of goods and tourists during the summer is the major entry route of this mosquito species.
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Introduction

In 1990, the Asian tiger mosquito, *Aedes albopictus* (Skuse, 1894) (Diptera; Culicidae), was found in the Port of Genova, Italy (Sabatini *et al.*, 1990). Since that first record, this mosquito has become established throughout Italy (Romi *et al.*, 2009; ECDC, 2009), spreading all over the country by passive transport and certainly by successive waves of further introductions (Romi *et al.*, 2006). This was supported by its adaptation to the winter conditions of the temperate areas (Romi *et al.*, 2009). To date, this species is distributed over large parts of the world and firmly established in the Americas, Caribbean, Africa, countries in the Mediterranean, including Turkey, Lebanon, Israel and Syria, and some countries of continental Europe from where it gradually moves northwards (Medlock *et al.*, 2012; Kraemer *et al.*, 2015).

The global dispersal of *Ae. albopictus* is facilitated by the transport of its eggs or larvae (Reiter & Sprenger, 1987; ECDC, 2012a) through the trade of goods such as used tyres (Knudsen, 1995) or lucky bamboo (*Dracaena* spp.) (Madon *et al.*, 2002; Scholte *et al.*, 2008).

Aedes albopictus is a peridomestic and anthropophilic species, that was responsible for the first European outbreak of chikungunya in the Emilia Romagna Region, Italy, in 2007 (Rezza *et al.*, 2007) and has been shown experimentally to be a competent vector of at least 26 arboviruses, including West Nile virus (WNV) (Paupy *et al.*, 2009; Fortuna *et al.*, 2015) and Zika virus (ZIKV) (Chouin-Carneiro, 2016; Di Luca *et al.*, 2016).

This note describes the occurrence of *Ae. albopictus* in three minor Italian islands in the Tyrrhenian Sea, where it has never been recorded before, namely the Island of Giglio, off the coast of Tuscany Region, the Island of Ventotene which is part of the Lazio Region, and the Island of Ustica, just off the coast of northern Sicily. Following the subsequent recording of the species on Lampedusa, Linosa (Pelagie Islands) and Pantelleria in the Sicilian Strait in 2015 (Romi *et al.*, 2016), this report represents the second study and record of the species on small Italian islands. In the Mediterranean Sea, *Ae. albopictus* has also been found on Maltese islands (Gatt *et al.*, 2009) where it is currently very abundant (ECDC, 2016), Mallorca, Ibiza and Minorca (Balearic Islands, Spain) in 2012, 2014 and 2015, respectively (Miquel *et al.*, 2013; Barceló *et al.*, 2015; Bengoa *et al.*, 2016).

Materials and Methods

The study was carried out on three minor Italian islands in the Tyrrhenian Sea, Giglio (Region of Tuscany), Ventotene (Region of Lazio) and Ustica (Region of Sicily) (Fig. 1) during three different surveys in summer 2016. These islands are characterised by a temperate climate with more rainfall in winter than in summer (en.climate-data.org,2017).

Giglio (42°21′56″N 10°54′06″E, 23.8 km^2 surface, 1,447 inhabitants, 61 inhabitants/km^2), belongs to the Tuscany Archipelago, located about 16 km from the mainland (Porto Santo Stefano). On Giglio, the average annual temperature is 15.8 °C, and the annual precipitation averages 518 mm (https://en.climate-data.org/search/?q=Giglio+campese). Three ovitraps were set in three different sites in the northeastern part of Giglio (Fig. 1): 1) Giglio Castello, an urban site located in a medieval village; 2) Arenella, a peri-urban site in a tourist village; and 3) Mare All'Arenella, another peri-urban site in a tourist village very close to the sea. Black plastic containers (14 cm in diameter and 12 cm in height) with an overflow hole, filled to two thirds with tap water (0.4 litre), were used as ovitraps; a Masonite® strip (15 cm long and 3 cm wide) was used as an oviposition support. The three ovitraps were placed in shady places, rich in vegetation in the courtyards of private homes from 5th-12th June and from 4th-10th July. Moreover, one BG-Sentinel mosquito trap (Biogents, Germany) baited with BG-Lure (Biogents, Germany) was operated at site 3, working for 48 hours within each of the two weeks.

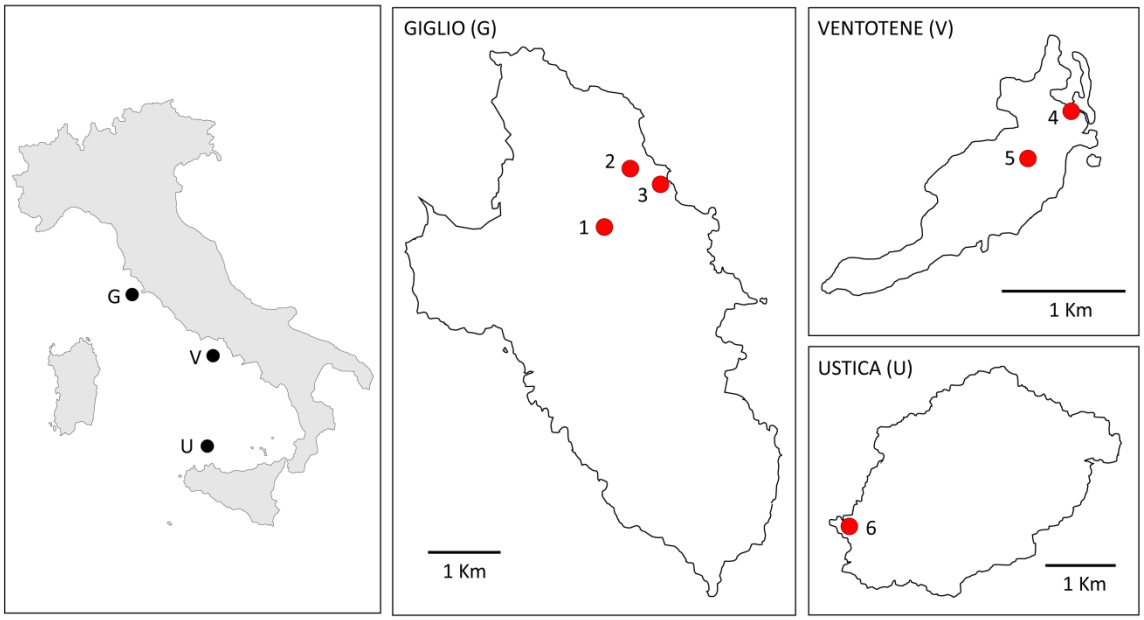

Figure 1: Location of the three sampled islands in the Tyrrhenian Sea (left), and specific geographic positions of ovitrap sites on the Island of Giglio (G), the Island of Ventotene (V) and the Island of Ustica (U).

Ventotene (40°48′N 13°26′E, 1.89 km^2 surface, 746 inhabitants, 317 inhabitants/km^2) belongs to the Pontino Archipelago, which is located 51.4 km from the mainland (Port of Formia). On this island, the average temperature is 15.5 °C and the annual average rainfall is 832 mm (https://www.ilmeteo.it/portale/medie-climatiche/Ventotene). Two ovitraps, as described above, were set at two different sites: 4) Calarossano and 5) Ventotene village, working for three days from 27th-29th September.

In Ustica (38°43′N 13°11′E, 8 km^2 surface, 1,359 inhabitants, 170 inhabitants/km^2), located about 66 km from the mainland (Port of Palermo), the average annual temperature is 17.8 °C and the annual average rainfall is 449 mm (https://en.climate-data.org/search/?q=Ustica). On this island, it was only possible to place one ovitrap in Punta Spalmatore locality (site 6) from 27th June-3rd July.

After the exposure of the ovitraps, the Masonite® strips were transported to the laboratory to examine the possible presence of aedine mosquito eggs; where present, eggs were counted and flooded for larval hatching. When the ovitraps contained larvae, live specimens were transported to the laboratory and reared until adulthood. The BG sentinel trap was emptied daily, and the collected mosquito specimens were stored dry.

In addition, water receptacles, such as manholes, buckets, pots etc., were inspected for mosquito developmental stages whenever possible. Larvae and pupae were collected, transported alive to the laboratory and reared until adulthood. Adult mosquito specimens were identified according to morphological keys (Schaffner *et al.*, 2001; Severini *et al.*, 2009).

Results

On the Island of Giglio, all ovitraps were positive for *Ae. albopictus*. In site 1, 82 eggs were found in June and 35 eggs in July; in site 2, 116 and 52 eggs were found in June and July, respectively; in site 3, no eggs were found in June, and 38 eggs were found in July. The BG-Sentinel trap collected one male and two female *Ae. albopictus* in June and one female in July. During the field activities, females of *Ae. albopictus* biting humans were observed repeatedly. From the eggs brought into the laboratory, 315 *Ae. albopictus* adults developed.

In Ventotene, site 4 ovitrap collected no eggs, but nearby this site, one *Ae. albopictus* female was captured while entering a house in the evening. At site 5, the ovitrap contained three eggs, and one *Ae. albopictus* female approached while the trap was emptied. After completing development in the laboratory, three adults of *Ae. albopictus* were identified. Moreover, numerous *Ae. albopictus* larvae were found in a paint bucket left in a pile of rubbish and filled with rainwater.

The only ovitrap placed on the Island of Ustica (site 6) collected 45 eggs within a week. From these, 38 *Ae. albopictus* developed. Moreover, during the stay on the island, several direct observations of *Ae. albopictus* biting activity on humans were carried out during day-time. The results of the field activities carried out on the three island are summarised in Table 1.

Discussion

In addition to the Balearic Islands and the Maltese Archipelago, the only further cases of islands colonised by *Ae. albopictus* in the Mediterranean Sea are represented by Sardinia and Sicily. In Sardinia, *Ae. albopictus* had been eradicated thanks to the intervention of the local public health agency in 1995 (Nuvoli & Pantaleoni, 2003) but the species reemerged in 2006 (Cristo *et al.*, 2006) and then spread across the whole island (http://ecdc.europa.eu/en/healthtopics/vectors/vector-maps/Pages/VBORNET_maps.aspx). Also in Sicily, which is very close to the mainland, *Ae. albopictus* is now widespread (http://ecdc.europa.eu/en/healthtopics/vectors/vector-maps/Pages/VBORNET_maps.aspx). As for similar settings like the Maltese Archipelago and the Balearic Islands (Gatt *et al.*, 2009; Bengoa *et al.*, 2016), it is difficult to speculate on when *Ae. albopictus* had arrived on the three islands, because people often only become aware of mosquitoes when annoyance takes place.

Table 1: *Aedes albopictus* specimens collected and observed during the field activities on the three islands.

Island	No. ovitraps	No. eggs	No. larvae collected in the field	No. mosquito traps	No. adults collected	No. adults observed	Larval breeding sites sampled
Giglio	3	323	0	1	3	≥ 20	0
Ventotene	2	3	≥ 500	0	1	1	bucket
Ustica	1	45	0	0	0	≥ 10	0

As an introduction route, the maritime route is the most likely pathway, also considering the absence of airports on the islands sampled. All three islands are popular with tourists, located very close to the mainland and easily accessible by maritime routes throughout the year. Transport ships and ferries heading for all three islands several times a day from May-December start from to the coasts of Tuscany, Lazio and Sicily, respectively, where *Ae. albopictus* is currently present. For the Island of Giglio, the largest of the three islands, the most likely route of introduction of *Ae. albopictus* is through vehicles carried by ferries, as this island is the only one among the three islands considered here where people are allowed to bring their private cars. Moreover, the Costa Concordia disaster in January 2012 was followed by three years of intense ship transport of equipment used for the recovery operation and also of technicians, workers, and journalists.

The availability of larval breeding sites on the islands is variable, depending mainly on rainfall. On Ustica and Giglio, water is additionally provided by desalination systems which are widely used for agricultural purposes. By contrast, Ventotene is supplied by water tanks, permitting a much more limited use of water. Potential larval breeding sites are therefore fewer and depend even more on rainfall.

In general, the data here reported confirm the high capability of *Ae. albopictus* in overcoming biogeographical barriers and in reaching islands. These considerations also raise concern for the spread of another invasive mosquito species, *Aedes* (*Stegomyia*) *aegypti* Linnaeus 1762, the primary vector of DENV, ZIKV and Yellow fever virus (YFV). Recently, *Ae. aegypti* acted as a vector of DENV on the Island of Madeira (ECDC, 2013). This species is currently not present in Italy (http://ecdc.europa.eu/en/healthtopics/vectors/vector-maps/Pages/VBORNET_maps.aspx), but was present in the last century (Toma *et al.*, 2011), and there is growing concern that it could become widely established again in the Mediterranean (ECDC 2012b; 2013), since it has expanded its distribution range in countries bordering the Black Sea, such as Russia, Georgia and Turkey (Akiner *et al.*, 2016; Ganushkina *et al.*, 2016).

As each island considered here shows peculiar features, the occurrence of *Ae. albopictus* deserves to be studied further, not only on each individual island but also at an archipelago level. In fact, the different amounts of annual rainfall, geology, water availability for domestic uses and housing types certainly influence the density of the local *Ae. albopictus* population. Further investigations aimed at understanding how these factors act on each island would be of considerable interest.

Conclusion

The occurrence of *Ae. albopictus* on minor islands of the Tyrrhenian Sea, as reported here for the first time, raises concern for health authorities. In the case of the introduction of a person viraemic for ZIKV, CHIKV or DENV into this tourist region, a scenario of local transmission could develop, putting a large number of people at risk. While chikungunya broke out already in Italy (Rezza et al., 2008), autochthonous cases of Zika and dengue have not been recorded. The number of imported cases of CHIKV (63), DENV (231) and ZIKV (45) from 2014-2016 (Italian Ministry of Health, 2016), however, are alarming.

Moreover, given the impact of passive transportation on the spread of *Ae. albopictus*, trade routes supporting the displacement of eggs and larvae should be controlled and reduced or even eliminated.

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