ENTOMOLOGY

Colonization by tiger mosquito (*Aedes albopictus* Skuse, 1894) of mountain areas over 600 m above sea level in the surroundings of Trento city, Northeast Italy

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Abstract

Originally from Southeast Asia, the tiger mosquito (*Aedes albopictus*) is now found almost everywhere in the world. Additionally, it spread throughout all of Northeastern Italy's cities, including Trento, and settled in the Alpine regions. The invasion of mountain regions in the Alps above 600 meters, which is generally thought to be the upper limit of *Ae. Albopictus*'s range, is confirmed for the first time in this note. We observed it in the summer of 2023 between 615 and 708 meters above sea level in the Trento region's surrounding mountains, specifically in the Montevaccino and Sopramonte localities. The mosquito was caught using ovitraps, and by raising the eggs to adulthood in a lab, the species identification was verified. This finding heightens worries about how tropical insect species, which are favored by global warming, may invade the mountains and affect tourism.

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Introduction

Aedes (*Stegomyia*) *albopictus* (Skuse, 1895) (Diptera: Culicidae), known as "the Asian tiger mosquito", is an invasive species native to Southeast Asia, widespread by now in all continents except Antarctica (Kraemer *et al.*, 2015) via human activities and active transportation (Gratz, 2004). It arrived in Europe in 1979 (Albania) and in a few decades invaded many countries including Italy, where it was reported for the first time in Genoa in 1990, probably imported from the United States together with tyres (Sabatini *et al.*, 1990). In the late '90s, it was recorded in the southern Trentino Province and today invaded all the lowland valleys (Lencioni *et al.*, 2018). Municipalities invest money every year to monitor its distribution and abundance, because of its medical interest and nuisance, annoying residents and tourists frequenting the capital city (Trento) and the surrounding mountains during summer. It is a vector of at least 22 arboviruses (Gratz, 2004; Schaffner *et al.*, 2013) including Dengue, Chikungunya and Zika with outbreaks occurred in Italy in the last decade (Barzon *et al.*, 2021). In Italy, the monitoring is regulated by the plan for the prevention, surveillance and control of arboviruses (PNA 2020-2025, Ministero della Salute, 2019) which requires the monitoring of mosquitoes below 600 meters above sea level (a.s.l).

Its rapid spread to mountain regions in temeprate climates is related (i) to the capacity of *Ae. albopictus* to lay diapausing eggs allowing them to survive in winter and (ii) to increasing temperature due to climate change. Winters are becoming milder and summers are increasingly hot even at altitudes higher than 600-700 m, where the probability of finding the tiger mosquito is considered medium-low (Reinhold *et al*., 2018). It was sporadically recorded at a higher altitude in Albania (at 1000 m a.s.l.; Tisseuil *et al.*, 2018) and in the Lazio region (Italy) (at 900 m a.s.l.; Romiti *et al.*, 2022). In fact, an average January temperature >0°C permits diapausing eggs to overwinter, an average annual temperature >11°C is required for adult survival and activity and a maximum summer temperature of 25-30°C is considered optimal for its development (Medlock *et al.*, 2015). Regarding precipitation, a total of 290-500 mm/year is known as the minimum value to maintain aquatic

habitats suitable for *Ae. albopictus* establishment (Eritja *et al.*, 2005).

Within this context, in 2023 our routine monitoring included two localities above 600 m of altitude in the surrounding mountains of Trento, Montevaccino and Sopramonte, where it was never recorded in the past (Lencioni *et al.*, 2023). Here many residents of the capital city have their second house and so there is heavy car traffic in summer causing a possible passive transport of adults (Probstl-Haider *et al.*, 2015). In fact, the dispersion capacity of this and other *Aedes* mosquitoes is rather low (200-300 m from the hatching site; Marini *et al.*, 2019). In addition, we hypothesized to have here a temperature suitable for *Ae. albopictus* survival, based on values recorded in the meteorological station of Centa, located at 805 m a.s.l. nearby Montevaccino. Specifically in 2022, at the Centa station, the temperature and precipitation have exceeded the values considered as the average and total limit for *Ae. albopictus* survival: 3.1°C in January, annual of 11.2°C, maximum summer of 26.6°C and 829 mm for the total precipitation. In addition, neighboring localities below 600 m a.s.l. in the Argentario and Bondone districts have been already infested by this species for several years (Figure 1; Lencioni *et al.*, 2023). The aim of this study was to intercept any eventual first outbreak of tiger mosquito in these areas and to verify the occurrence of any other *Aedes* mosquito using the same oviposition place as *Ae. albopictus*.

Materials and Methods

Aedes eggs were caught using four ovitraps in Montevaccino and Sopramonte from early July to the end of August 2023 (Figure 1, Table 1). The ovitrap location was at an average altitude of 696 m a.s.l. in Montevaccino and of 638 m a.s.l. in Sopramonte. Based on Centa meteorological station, at 805 m a.s.l. (https://www.meteotrentino.it), the average annual and the maximum summer temperature have been increasing in the last 10 years, from 10.1 to 10.9°C and from 24.3 to 25.2°C respectively.

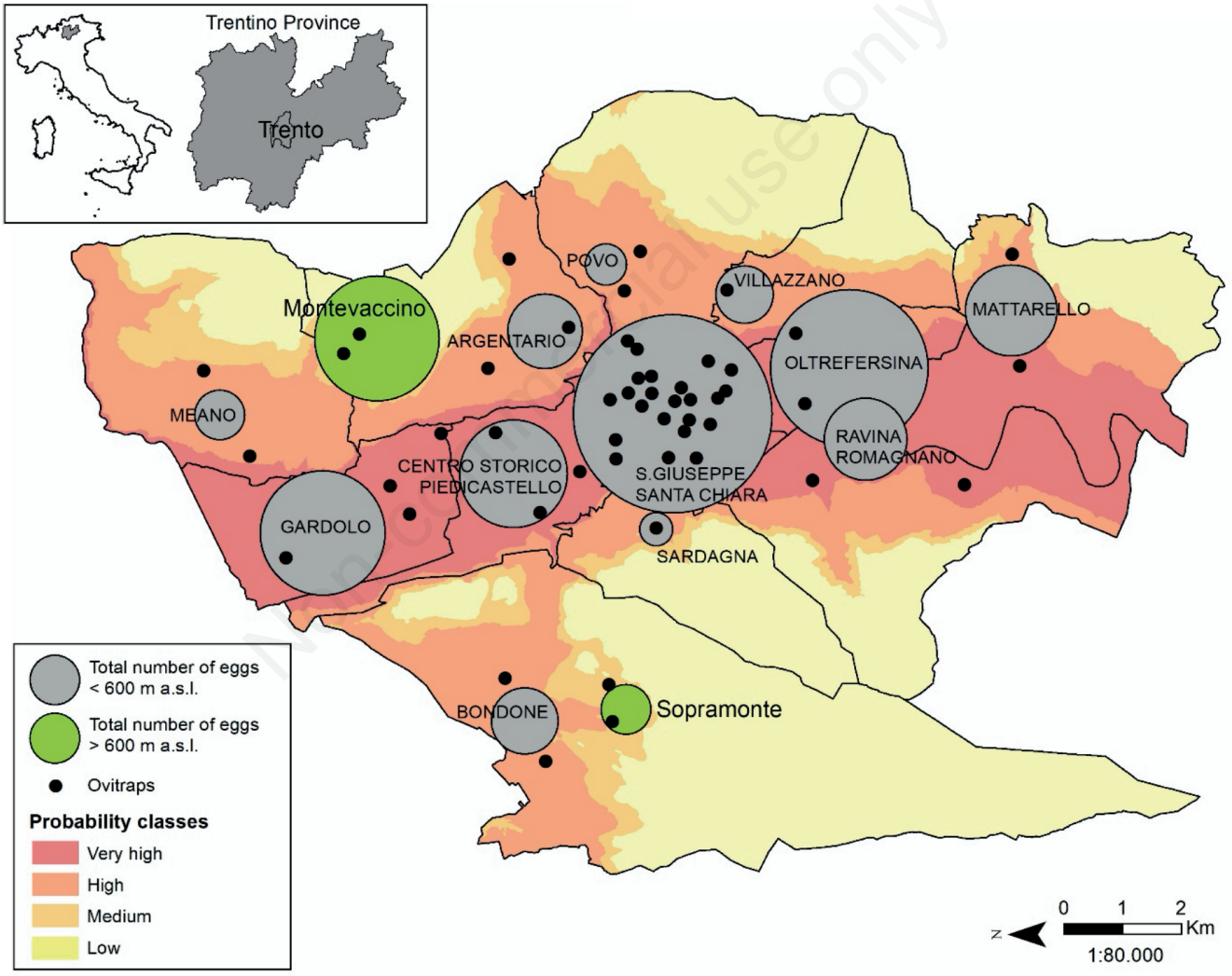

Figure 1. Map of ovitraps distribution (black points) and eggs abundance of *Ae. albopictus* in the Trento municipality area (Trentino region, Northeast Italy) in July-August 2023. Green spheres refer to ovitraps located in Montevaccino and Sopramonte localities (>600 m a.s.l.); grey spheres refer to ovitraps located in the eight monitored Trento districts <600 m a.s.l. The size of the spheres is proportional to the total number of eggs found in the ovitraps, from a minimum of 48 eggs (in Sardagna) to a maximum of 12,077 eggs (in San Giuseppe-Santa Chiara). The colored bands (from yellow to red) represent the probability classes of the presence of *Ae. albopictus* as reported in the Provincial Guidelines for mosquito control (DGP n. 1049 15 June 2018: https://www.aedescost.eu/sites/default/files/2023-01/IT_Trento-Piano_Control_ZanzaraTigre_ProvTrento-2018.pdf).

Table 1. Altitude and geographical coordinates (EPSG:4326 - WGS84) of the ovitrap locations and total number of adults of the three *Aedes* species flickered in the laboratory from the eggs collected with the ovitraps.

	Ovitrap	Altitude (m a.s.l.)	Latitude (°N)	Longitude (°E)	*Ae. albopictus*	*Ae. koreicus*	*Ae. geniculatus*
Montevaccino	MV1	708	46.108373	11.1420337	316	1	2
	MV2	683	46.1108789	11.1378287			
Sopramonte	S1	615	46.0711724	11.0551177	222	19	0
	S2	660	46.0715766	11.0633555			

The annual precipitation has remained above 800 mm in the last decade, with a slight decrease in the last 3 years than in 2012-2014 (from 1,738 to 1,038 mm).

The employed ovitrap was a small black plastic container (height =12 cm, diameter =8 cm, volume =400 ml) with a hole of two cm from the edge to prevent overfilling, mimicking the preferred natural and artificial breeding sites for *Ae. albopictus* (tree-holes, rock-holes and small man-made containers). The container was filled for two-thirds with water and a wood or masonite rough paddle (width =3 cm; thickness =0.3 cm; length =13 cm) was inserted for females to lay eggs on. Eggs were collected every fortnight from 18 July to 29 August 2023, for a total of 4 collections and 16 laying paddles (Table 1). The eggs from the two traps, from each location and date, were reared together in 500 mL glass beakers filled with tap water and covered with a net (100 um mesh size) at a room temperature of 25±1°C under natural photoperiod. The rearing beakers were checked every 2-3 days. Adults were identified according to Severini *et al.* (2022).

Results, Discussion and Conclusions

A total of 3746 eggs were collected, of which 3004 in Montevaccino and 742 in Sopramonte. In Figure 1, the egg abundance found in these localities was compared with that one recorded in the other sites monitored in Trento districts below 600 m of altitude. A total of 560 adult specimens flickered, of which 319 were from Montevaccino eggs and 241 from Sopramonte eggs. Therefore, the hatching fraction was 11% and 32% in Montevaccino and Sopramonte respectively, as average comparable with the fraction of hatching and emergence found by Marini *et al*. (2019), *i.e.*, 19% at 25°C for *Ae. albopictus*. The adult identification confirmed the presence of *Ae. albopictus* in the two localities, along with *Aedes (Finlaya) koreicus* (Edwards, 1917) and, in Montevaccino, *Aedes (Finlaya) geniculatus* (Olivier, 1791). The duration of the entire development from egg to adult at 25±1°C lasted 10-15 days for *Ae. albopictus* and about 30 days for the other two species. These values are comparable with those found by Marini *et al*. (2019) for *Ae. albopictus*, *i.e.*, 16.5 days at 25°C.

What we did not expect was the dominance of *Ae. albopictus* on the other two species. It represented 99% and 92% of the flickered adults in Montevaccino and Sopramonte respectively. In fact, *Ae. koreicus* is another invasive mosquito species originating from East Asia that is well adapted to urban settlements. However, as opposed to *Ae. albopictus*, the cold season is not a limiting factor to the spread of *Ae. koreicus* which is *de facto* more typical of mountain locations than *Ae. albopictus* (Marini *et al.*, 2019). It can tolerate the cold winter temperature and the first larvae are found early in spring (Montarsi *et al.*, 2013). In addition, the coexistence between *Ae. koreicus* and *Ae. albopictus* is possible but not common. When occurring, generally *Ae. albopictus* prevails but not at high altitudes (Montarsi *et al.*, 2013). Our result is probably due to the late colonization by *Ae. koreicus* with respect to *Ae. albopictus*, being recorded in Europe only in 2008 (Versteirt *et al.*, 2012) and in the Trentino Province with established populations in mountain localities only in 2016-2018 (*e.g.*, Cognola and Villamontagna nearby Montevaccino) (https://vettoritrentino.it/). Unlike *Ae. albopictus* and *Ae. koreicus,* which are invasive alien species, *Ae. geniculatus* (Olivier, 1791) is an indigenous mosquito species; the biology of this species is not well known but it is easily found in forest areas (Fruh *et al.*, 2020), and the larvae are often found in tree cavities (Bradshaw *et al.*, 1986). Little information on *Ae. koreicus* and *Ae. geniculatus* vector competence is available. Recent studies confirmed that both have a vector competence at least for the Chikungunya like *Ae. albopictus* (Ciocchetta *et al.*, 2018; Prudhomme *et al.*, 2019). This means that the risk of transmission of at least this virus will be extended in this newly colonized area, *i.e.*, in mountain areas previously considered at negligible risk of human outbreaks. Considering the consistent number of eggs collected in Montevaccino and Sopramonte, higher than that collected in neighboring areas at <600 m a.s.l., we can argue that this species has been established in the two localities. This finding will increase the concern about the invasion of the mountains in the Alps of these species which the public administration and tourism structures will be dealing with in the next future.

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