First record of Aedes japonicus in Liguria region, Northwest Italy

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Summary
Aedes japonicus is an invasive Asian mosquito species, and to date it is widespread in many European countries. In Italy, it was first recorded in 2015 at the Austrian border and it then spread throughout the Northeast of the country. In 2019, it was also identified in Piedmont region, near the Swiss border. In the framework of the Italian program for prevention, surveillance, and response to Arboviruses, from June to November 2021, biweekly entomological surveillance was performed in the Liguria region (Northwest Italy). The collected mosquitoes were morphologically and genetically identified and molecularly analysed for the detection of West Nile and Usutu viruses. Six female mosquitoes, trapped on the 6th of July 2021 using a gravid trap in Albenga (Savona province), were morphologically identified as Aedes japonicus and the identification was genetically confirmed. The pool tested was negative for the presence of West Nile and Usutu viruses. The detection of Aedes japonicus was performed in a coastal area characterized by the presence of many floriculture activities. Considering the distance from the established Aedes japonicus mosquito populations in Italy and other European countries, this could represent an independent introduction in this country.

The invasive species Aedes japonicus (Theobald, 1901) is native to East Asia and the far East (Tanaka et al., 1979; Kampen et al., 2014). From the 90’s it has spread in New Zealand (Laird et al., 1994), the USA (Peyton et al., 1999) and in Europe, where, after its first detection in the north of France in 2000 (Schaffner et al., 2003), this species was observed in different European countries, such as Belgium (Versteirt et al., 2009), Switzerland, Germany (Schaffner et al., 2009), the Netherlands (Ibañez-Justicia et al., 2009), Austria, Slovenia and Hungary (Seidel et al., 2016a), Croatia (Klobucar et al., 2019), and Spain (Eritjia et al., 2019). It was also reported recently in Bosnia and Herzegovina and Serbia (Janssen et al., 2020). This species was identified for the first time in Italy in 2015 at the Austrian border (Seidel et al., 2016b) and then spread in the Northeast of the country, colonizing two regions in few years (Montarsi et al., 2016). In 2019, Aedes japonicus was also isolated in Piedmont region (Northwest Italy), about 20 kilometres from the Swiss border, and in the two following years this species spread all over the region (Mosca et al., 2022). The source of introduction of this species in European countries is not clear, a connection with the tyre trade importation and ground transport has been hypothesised (Medlock et al., 2012).

Aedes japonicus is an experimentally proven vector for the transmission of flaviviruses such as Japanese Encephalitis virus (Huber et al., 2014), Chikungunya, Dengue (Schaffner et al., 2011) and Zika viruses (Jansen et al., 2018); its vector competence for West Nile virus (WNV) is debated. A transmission...
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The genetic analyses confirmed the morphological identification. Regarding the 
COI gene, a 100% identity was found with the sequences of Aedes japonicus deposited in GenBank.

The same result was confirmed with the ND4 gene analysis, where the similarity was 98.9%. Furthermore, the sequences showed the characteristic mutation in the position 181, which, according to Cameron et al. (Cameron et al., 2010), represents a diagnostic site: T for Aedes koreicus, G for Aedes japonicus, A for the other Aedes species.

Representative sequences were submitted to GenBank with ON911330 accession number for the COI sequence and ON933815 for the ND4 sequence. Phylogenetic analyses confirmed the identification of Aedes japonicus (Fig. 1).

Figure 1. a: Torax. Scutum dorsal side: stripes of golden scales, submedians long; b: Last segments of the third leg. Segments IV and V black.

Both strands using BigDye Terminator v3.1 Cycle Sequencing Kit (ThermoFisher Scientific, Waltham, MA) and each obtained consensus sequence was compared with sequences deposed in GenBank. A similarity ≥98.0% was considered as cut off for identification at the species level. Moreover, phylogenetic analyses were performed with MegaAlign sequence alignment software (DNASTAR Inc., Lasergene package) to generate a phylogenetic tree with a bootstrap analysis to evaluate the statistical significance of the nodes. A value ≥70 corresponds to a statistical significance value ≥95% at the node.

Following the national surveillance plan, female specimens of the same mosquito species from each site were pooled to test for the presence of WNV and USUV. RNA was extracted using a commercial kit (Rneasy Mini Kit 250, QIAGEN, Valencia, CA) and analysed by a multiplex real-time reverse transcription-PCR for the simultaneous detection and differentiation of WNV Lineages 1 and 2 (Del Amo et al., 2013), and by a real-time RT-PCR specific for USUV detection (Cavrini et al., 2011).

Six female specimens of mosquito collected in Albenga, in the province of Savona (44.067169 N, 8.158698 E) using a gravid trap, on the 6th of July 2021, were individually morphologically identified as Aedes japonicus (Fig. 1).

rate for WNV even higher than for Culex pipiens was observed in a study conducted in laboratory conditions (Turell et al., 2001), while in another one A. japonicus seemed refractory to the infection with WNV (Huber et al., 2014). However, Aedes japonicus collected in the USA have been found infected with WNV (De Carlo et al., 2020).

In Italy a national plan, named “Plan for prevention, surveillance, and response to Arboviruses”, is enforced by the Ministry of Health (Italian Ministry of Health website).

Among the objectives of this plan are the surveillance of new invasive species, potential vectors of arboviruses, and the surveillance of WNV and Usutu virus (USUV) circulation in mosquitoes. This plan provides for the identification on the national territory of sites for entomological and virological surveillance, in the period of maximum circulation of mosquitoes. Such sites are identified on the basis of the risk of WNV and Usutu viruses (USUV) circulation and of the risk of new invasive species introduction. The Liguria region is characterized by many important commercial and touristic ports, and also by various floriculture activities and markets, possible sources of introduction of invasive mosquito species. For these reasons, entomological surveillance is fundamental for their early detection and monitoring.

The aim of this work is to report the identification of Aedes japonicus in Liguria in the framework of the above-mentioned surveillance plan.

A biweekly surveillance was performed in Liguria region (Northwest Italy) from June to November 2021. The traps were set up for both WNV-USUV surveillance and for invasive mosquitoes monitoring. Adult mosquitoes were trapped using CO2-lure-baited BG-sentinel traps or gravid traps, both types working overnight, in 20 sites. After the collection, the mosquitoes were transported refrigerated to the entomological laboratory where they were morphologically identified using entomological keys (Severini et al., 2007, Cameron et al. 1946) were used for the identification of invasive species. In case of doubtful identification, single specimens were identified by molecular analysis: after homogenization in phosphatase-buffered saline (PBS), DNA was extracted using a commercial kit (ReliaPrep™ gDNA Tissue MiniPrep System – Promega, Madison, WI). Then, a portion of two different mitochondrial genes, cytochrome oxidase subunit I (COI) gene and nicotinamide adenine dinucleotide dehydrogenase subunit 4 (ND4) gene, were amplified by PCR following previously described methods (Dawnay et al., 2007, Cameron et al., 2010). The PCR products were sequenced on both strands using BigDye Terminator v3.1 Cycle Sequencing Kit (ThermoFisher Scientific, Waltham, MA) and each obtained consensus sequence was compared with sequences deposed in GenBank. A similarity ≥98.0% was considered as cut off for identification at the species level. Moreover, phylogenetic analyses were performed with MegaAlign sequence alignment software (DNASTAR Inc., Lasergene package) to generate a phylogenetic tree with a bootstrap analysis to evaluate the statistical significance of the nodes. A value ≥70 corresponds to a statistical significance value ≥95% at the node.

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showing clustering of the obtained sequences with other Ae. japonicus sequences from GenBank for both gene targets (Fig. 2). The pool tested was negative for WNV and USUV.

For the first time the invasive species Ae. japonicus was identified in Liguria region, Northwest of Italy, six years after its first detection in the region and in a site hundreds of kilometres away from its spreading area in the Northeast of Italy, and also from the other recent Italian finding in the Piedmont region. The described record could thus represent an independent introduction in a territory characterized by intensive floriculture activities, importing plants and flowers from many parts of the world. However, this hypothesis needs to be considered with caution as it was not possible to demonstrate the exact time and route of introduction. During the 2021 monitoring season, this was the only finding of Ae. japonicus. Also in other European countries, such as Germany, multiple independent introductions of this species have been observed in different areas and time (Schneider 2011, Zielke et al., 2015, Reuss et al., 2018). The southernmost detections reported for this species in Europe are in Spain, in the Asturian region (Eritjia et al., 2019), and in Eastern countries such as Croatia, Bosnia and Herzegovina and Serbia (Janssen et al., 2020). The site of the present detection is almost at the same latitude but it is characterised by different climate conditions.

The Liguria region has a Mediterranean climate, characterised by hot, dry summer and mild, wet winters. In particular, in the city of Albenga, the mean daily temperature ranges between 3-12°C during winter and between 19-29°C during summer. The optimal temperature for Ae. japonicus life-cycle has been experimentally investigated. An optimal female survival temperature at 25°C and minimal at 7°C have been proven. The larvae are capable to hatch after exposure to 5°C water temperature and can survive at low temperatures for a long period. The hatching success decreases drastically at 0°C and -5°C (Reuss et al., 2018). Thus, considering the winter temperature of the area, and the diapause capability of Ae. japonicus (Krupe et al., 2021), it is possible to expect an overwintering. Ae. japonicus is considered a cold-tolerant species and in North America; it has been hypothesised that the expansion to southern latitudes is limited by temperatures regularly exceeding 30-35°C (Kaufman et al., 2014). Moreover, experimentally the development to female adults has been observed at maximum 31°C (Reuss et al., 2018). The low and high temperatures are limiting for Ae. japonicus and it is probably also for this reason that it is present only in some European countries. Moreover, Cunze et al. (Cunze et al., 2016) modelled the possible future ecological niches for Ae. albopictus and Ae. japonicus in Europe, considering climate changes and environmental characteristics. The Mediterranean regions of Italy, characterised by a warmer climate, with summers that are becoming hotter and hotter over time, are not considered suitable for the presence of Ae. japonicus, that is less able to expand in warmer conditions compared to Ae. albopictus (Kaufman et al., 2014).

In the Liguria region, other Aedes invasive mosquito species occurred, such as Ae. albopictus, widely present, and Ae. koreicus was detected for the first time in 2015, then it has become endemic in Genova province (Ballardini et al., 2019). It would be interesting to observe the future distribution of Ae. japonicus in the Liguria region, which to date is one of the southernmost European sites of detection, and its distribution overlaps with other Aedes species. The province of Savona, where the pool was collected, is considered by the above-mentioned national plan on Arboviruses, to have a low level of risk for the circulation of WNV, because the virus has not been detected in the past five years. However, the circulation of the virus has been observed in the neighbouring province of Genova, an area with the same climate and environmental conditions. So, the future possible spreading of this species as potential vector could represent a risk also for the spread of arboviruses. Unfortunately, no control measures have been taken to eradicate this species. The entomological surveillance included in the national plan for prevention, surveillance, and response to Arboviruses allowed to detect Ae. japonicus in the Liguria region, in the northwest of Italy, which could represent an independent introduction in the Country. Data on the following monitoring activities will help in understanding if Ae. japonicus is becoming endemic in the area and if it is spreading to other sites.
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References


