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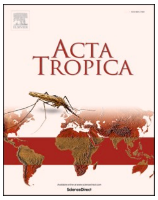

Assessing the distribution of invasive Asian mosquitoes in Northern Italy and modelling the potential spread of *Aedes koreicus* in Europe

Irene Arnoldi [a,b,c,d], Agata Negri [a,b], Laura Soresinetti [a,c], Mattia Brambilla [e], Davide Carraretto [c,d], Fabrizio Montarsi [f], Paolo Roberto [g], Andrea Mosca [g], Diego Rubolini [e], Claudio Bandi [a,b], Sara Epis [a,b,*], Paolo Gabrieli [a,b,*]

[a] *Department of Biosciences and Pediatric Clinical Research Center "Romeo ed Enrica Invernizzi", University of Milan, Milan 20133, Italy*
[b] *Italian Malaria Network, Inter University Center for Malaria Research, University of Milan, Milan 20133, Italy*
[c] *Department of Biology and Biotechnology, University of Pavia, Pavia 27100, Italy*
[d] *University School of Advanced Studies Pavia, IUSS, Pavia 27100, Italy*
[e] *Department of Environmental Science and Policy, University of Milan, Milan 20133, Italy*
[f] *Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro 35020, Italy*
[g] *Istituto per le Piante da Legno e l'Ambiente, I.P.L.A. S.p.A., Turin 10132, Italy*

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ABSTRACT

In the last decade, *Aedes koreicus* and *Aedes japonicus japonicus* mosquitoes, which are competent vectors for various arboviruses of public health relevance, colonised Italy and other European countries. Nevertheless, information about their current and potential distribution is partial. Accordingly, in this study four regions of Northern Italy (Lombardy, Liguria, Piedmont and Aosta Valley) were surveyed during 2021 for the presence of eggs, larvae and pupae of these two invasive species. We found evidence for a widespread presence of *Ae. koreicus* in pre-Alpine territories of Lombardy and Piedmont. Larvae from the invasive subspecies of *Ae. j. japonicus* were also collected in the same geographic areas, though they were less frequent. Occurrence data from this study and results from previous monitoring campaigns were used to generate a Maxent model for the prediction of habitat suitability for *Ae. koreicus* mosquitoes in Northern Italy and the rest of Europe. Peri-urban areas located in proximity to forests, pastures and vineyards were revealed as highly suitable environments for colonisation by this invasive species. Maps of the potential distribution also suggest the presence of further suitable areas in currently uncolonized countries. We conclude that this invasive mosquito species has the potential for a broad expansion at the European level in the coming decades.

1. Introduction

Invasive mosquitoes are an emerging problem, highly relevant to public health (Schaffner et al., 2013a), considering that most of these mosquitoes are potential vectors for a variety of pathogens, including viruses and filarial nematodes. The intense international travels and trades, part of the globalisation phenomenon (Schaffner et al., 2013a), together with climatic and environmental changes and landscape perturbations, favoured the establishment and adaptation of invasive mosquito species to new areas (Marcantonio et al., 2016; Medlock et al., 2012).

Notably, the vast majority of alien mosquito species belong to the *Aedes* genus: *Aedes notoscriptus*, the Australian backyard mosquito, is establishing in California (Metzger et al., 2022); *Aedes aegypti*, the yellow fever mosquito, has colonised the tropic and subtropic areas worldwide (Kamal et al., 2018); *Aedes albopictus*, the Asian tiger mosquito, is now found in all continents, except Antarctica (Sherpa et al., 2019).

Aedes koreicus and *Aedes japonicus japonicus*, whose home range occupies temperate regions of far East Asia, have recently been reported in various areas of Europe, as shown by ECDC distribution maps of both species (ECDC, 2022a; ECDC, 2022b) ; these two mosquitoes can tolerate cold temperatures and are supposed to disperse and overwinter as drought-resistant eggs, similarly to other aedine mosquito species

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* Corresponding authors at: Department of Biosciences and Pediatric Clinical Research Center "Romeo ed Enrica Invernizzi", University of Milan, Milan 20133, Italy.

E-mail addresses: sara.epis@unimi.it (S. Epis), paolo.gabrieli@unimi.it (P. Gabrieli).

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(Medlock et al., 2012).

Ae. koreicus is native to Korea, Japan, Northern China and Southern areas in far-east Siberia (Hohmeister et al., 2021). In Europe, it has so far been reported in specific areas in Belgium (2008), in Italy (2011), at the Swiss-Italian border and in Slovenia and European Russia (2013), in Southern (2015) and Central Germany (2016), in Hungary (2016), in Austria (2018) (Ganushkina et al., 2016; Negri et al., 2021) and in the Netherlands (2022) (Teekema et al., 2022). In Italy, *Ae. koreicus* mosquitoes were initially found in the district of Belluno (Veneto region) in 2011 (Capelli et al., 2011), and its increasing expansion in this area has recently been described (Gradoni et al., 2021). The presence of this species was also reported in Genoa (Liguria region, 2015; Ballardini et al., 2019) and in three different districts of the Lombardy region, namely Como in 2013 (Suter et al., 2016), Sondrio in 2015 (Montarsi et al., 2015) and Bergamo in 2020 (Negri et al., 2021).

Ae. japonicus is a complex of four morphologically similar subspecies, but only *Ae. j. japonicus* expanded from its native area: Japan, Korea, South-Eastern Siberia, and China, including Hong Kong and Taiwan (Kampen and Werner, 2014). In Europe, this subspecies was detected in Belgium (2002), in Switzerland and Germany (2008), in Slovenia and Austria (2011), in Hungary and in the Netherlands (2012), in Croatia and in France (2013), in Italy and Liechtenstein (2015), in Bosnia Herzegovina and Serbia (2017), in Spain and Luxembourg (2018) and in Romania and Slovakia (2020) (Čabanová et al., 2021; Horváth et al., 2021; Janssen et al., 2020; Koban et al., 2019; Smitz et al., 2021). In Italy, *Ae. j. japonicus* was first detected in 2015 in the Friuli Venezia Giulia (FVG) region at the border with Austria (Seidel et al., 2016). Subsequently, this invasive subspecies expanded to many other areas of FVG and Veneto regions (Gradoni et al., 2021; Montarsi et al., 2019).

Currently, the origin and the importation routes to Europe of these novel invasive species are not known (Kampen and Werner, 2014). Hypothesis about dispersal patterns focused mainly on *Ae. j. japonicus*, rather than *Ae. koreicus,* probably because of its wider distribution worldwide and earlier reports outside its native range. In general, *Aedes* invasive mosquitoes are thought to spread across countries and continents mainly through intercontinental transport of used tires, machineries, and vehicles (Derraik, 2004). This mode of dispersal has indeed been largely responsible for the spread of *Ae. albopictus* (Medlock et al., 2012), and plausibly also for that of *Ae. j. japonicus.* The displacement of tires was indeed associated with the initial introduction of this subspecies in the USA, New Zealand, and Europe (Horváth et al., 2021; Kampen and Werner, 2014). Trade of ornamental plants should also be investigated as an additional route of entrance for this mosquito in novel countries, considering that Lucky bamboo was implicated in the importation of *Ae. albopictus* in California and in the Netherlands (Medlock et al., 2012). Moreover, tracing back the way of importation of *Aedes* mosquitoes is complicated by the possibility of multiple introduction events which have already been observed for both *Ae. albopictus* (Manni et al., 2017, 2015) and *Ae. j. japonicus* (Huber et al., 2014; Janssen et al., 2020; Kampen and Werner, 2014; Koban et al., 2019; Smitz et al., 2021).

After the initial introduction in a novel region, *Ae. j. japonicus* spread passively through land-based trade, e.g. vehicle transport (Koban et al., 2019). Additionally, active expansion along river corridors, originally suggested to have occurred in the USA (Kampen and Werner, 2014), was then proposed as a possible way of dispersion of the subspecies in Austria and Slovenia countries (Kalan et al., 2017). Despite its low density in its native range, this subspecies quickly invaded multiple areas and established populations have been observed in Europe (e.g., Northern Germany and Belgium populations) (Huber et al., 2014; Kaufman and Fonseca, 2014; Koban et al., 2019).

On the contrary, *Ae. koreicus* expansion rate appears slower in several European countries: the populations in Germany and Belgium remained stationary and restricted to their early introduction areas. However, this trend is not observed in Italy (Hohmeister et al., 2021).

Early detection of invasive mosquito species and efficient monitoring is fundamental for the application of rapid control actions (Schaffner et al., 2013b). Despite the increasing number of reports of *Ae. koreicus* and *Ae. j. japonicus* in Europe, knowledge about their distribution needs to be improved in a widespread manner. In this regard, in this study we report the results of the survey activity carried out during summer and autumn 2021 in North-Western Italy, aiming to assess the current abundance of the above-mentioned invasive species in this area. Namely, our study highlights the massive diffusion of *Ae. koreicus* in many districts of the Lombardy region (Bergamo, Brescia, Sondrio, Lecco, Como) and of the Piedmont region (Asti, Turin, Alessandria). Moreover, we confirm the establishment of this mosquito species in the Liguria region (Genoa city) and its (at least apparent) current absence in Aosta Valley. Additionally, we report *Ae. j. japonicus* species in the Piedmont and, for the first time, in the Lombardy regions. Being *Ae. koreicus* the most widespread and with the larger population size in Italy, occurrence data for this species were used to realise a Maximum Entropy (Maxent) model for the prediction of habitat suitability in Northern Italy as well as in the rest of Central and Southern Europe.

2. Materials and methods

2.1. Mosquito surveys and collection

Surveys were carried out during summer and autumn 2021 (June-November) in four administrative units, i.e., regions of Northern Italy (EU NUTS2 codes: ITC1, ITC2, ITC3, ITC4). Particularly, we screened Alessandria, Asti, Biella, Cuneo, and Turin districts (Piedmont region, ITC-1), Aosta district (Aosta Valley region, ITC-2), Genoa district (Liguria region, ITC-3) and Bergamo, Brescia, Como, Lecco, Pavia, and Sondrio districts (Lombardy region, ITC-4). The study area shows a higher human population density (282.2 inhabitants/km^2) than the mean of the European Union (109.0 inhabitants/km^2) (Eurostat Data Browser, 2019). Given this population density, the area is characterised by intense agricultural, commercial, and industrial activities. Geographically, these regions are characterised by the presence of territories belonging to the Po River Valley and by the presence of the Alps to the north and to the west side.

Surveys consisted in opportunistic samplings. Indeed, they were planned based on several factors, such as former reports of the presence of the species of interest in an area. As previously stated, in the Lombardy region *Ae. koreicus* was found in the province of Como (Suter et al., 2016), Sondrio (Montarsi et al., 2015) and Bergamo (Negri et al., 2021). In the Piedmont region, particularly in the districts of Asti and Turin, *Ae. koreicus* and *Ae. j. japonicus* mosquitoes were previously observed by the IPLA regional institute in 2020 (unpublished data). Finally, in the Liguria region, *Ae. koreicus* was reported in 2015, in the city of Genoa (Ballardini et al., 2019). Moreover, a preliminary analysis of the territory, based on personal knowledge and using Google Earth software (Google Earth, 2005, available at: https://www.google.com/earth/download/ge/), was performed. Particularly, economic and social factors, like the location in poorly urbanised areas in proximity to pastures and forests, and environmental features, such as the elevation (generally higher than 400 m a.s.l.), were considered. Additionally, reporting from citizens and public institutions was included.

Breeding sites were detected in private and public gardens, garden centres, cemeteries, nature reserves and streets. Each accessible breeding site found in a chosen municipality, i.e., a man-made or natural water container, was checked for the presence of mosquito eggs (i.e., *Culex* sp. egg rafts), larvae, and pupae, which were collected using a spoon and carried to the insectarium in plastic bottles for adult emergence and classification. Adult mosquitoes were occasionally collected in the proximity of the breeding sites using an insect aspirator. All breeding sites were photographed and geo-referenced in decimal degrees, while the altitude of each location was determined using Google Earth software (Google Earth, 2005). Moreover, each breeding site was classified according to its location (private garden, street, cemetery etc.)

and features (small or big water container, tire, fountain etc.). Maps showing the screened municipality and the presence of *Ae. koreicus* and *Ae. j. japonicus* mosquitoes were generated with QGIS 3.18 software (QGIS.org, 2021, available at: http://www.qgis.org).

2.2. Morphological identification

Mosquito larvae were reared in the insectarium under standard conditions (25°C, 50% humidity), until adult development. Larvae and adult mosquitoes were identified under a stereomicroscope, following previously described morphological keys (Cameron et al., 2010; Farajollahi and Price, 2013; Negri et al., 2021; Romi et al., 1997; Severini et al., 2009). Particularly, identification of larvae was carried out when they did not complete the development during rearing.

2.3. Molecular identification

To confirm the morphological identification, molecular identification was performed for some samples for which the former was ambiguous. The DNA was extracted from the whole body of individual mosquitoes using a commercial kit (Monarch® Genomic DNA Purification Kit, New England BioLabs). One mitochondrial locus, i.e., the cytochrome oxidase I (*COI*), was amplified by Polymerase Chain Reaction (PCR) using LCO1490 and HCO2198 primers (Folmer et al., 1994) with a modified thermal profile (94°C 3'; 5 cycles 94°C 30'', 45°C 30'', 72°C 1'; 35 cycles 94°C 30'', 51°C 1', 72°C 1'; 72°C 10', 4°C 15'). Subsequently, PCR products were visualised on an agarose gel, purified and sequenced (Eurofins Genomics, Ebersberg, Germany). Sequences were compared with those available in GeneBank database using nucleotide Basic Local Alignment Search Tool (BLAST, 2021, available at: https://blast.ncbi.nlm.nih.gov/Blast.cgi). Finally, sequences were edited with BioEdit software (Hall, 1999) and deposited in GeneBank with the codes OM671292 – OM671311.

2.4. Modelling the distribution of Aedes koreicus

A Species Distribution Model (SDM) was built for *Aedes koreicus* based on a maximum entropy, presence-background, approach. Many different approaches have been proposed to build SDMs; they are all based on spatially explicit comparisons between occurrence records of the target species and some descriptors of the environment, but can rely on different assumptions and algorithms, which could potentially lead to rather different outcomes. Many works have compared results obtained with different methods, including model ensemble. Here, considering the non-standardized nature of our data, we used Maxent to build the distribution model (Phillips et al., 2006), which is preferable over other approaches when dealing with presence-only data (e.g. Elith et al., 2011; Grimmett et al., 2020), and had been shown to perform better than other methods when extrapolating over different areas (e.g. Brambilla et al., 2022). We therefore preferred to predict suitability over new areas by extrapolating predictions based on a single, but carefully parametrized algorithm (Hao et al., 2019; Kaky et al., 2020). Occurrence data were derived from our own surveys (**Table S1**), complemented with precise locations available from previous monitoring in Veneto and Friuli-Venezia Giulia regions (Gradoni et al., 2021). As potential predictors of mosquito occurrence, we considered selected bioclimatic, topographic and land-use/land-cover (hereafter LULC) variables (**Table S2**). All variables were computed as average value (climate, topography) or proportional cover (LULC) within 1 km x 1 km cells of a regular grid superimposed to the study area (and expanding over southern and central Europe to allow extrapolation to other regions). Multiple observations within the same 1-km^2 cell were considered as a single record. To appropriately reflect the environmental conditions sampled by the data collection, we placed background points (10028, one per cell) within a 4-km buffer around mosquito locations (cf. Brambilla et al., 2020). To develop generalizable models and explicitly check their robustness, we subdivided presence data into four different, spatially independent, data subsets, by means of the "checkerboard 2" method of the R package ENMeval (Muscarella et al., 2014), with 100-km^2 blocks aggregated in tetrads (aggregation factors: 10, 2). We then used data from three of the four partitions for model training ($N = 155$), while the fourth ($N = 50$) was kept as a test dataset.

We removed all the variables poorly represented in the sample region and performed a correlation test between the remaining ones. Only one pair of variables showed a high ($r > |0.7|$) correlation, annual mean temperature, and slope; the latter was therefore left out from the model as we regarded it as less ecologically informative. We then performed an AICc (Akaike's Information Criterion corrected for a small sample size)-based selection of the main model parameters, working with only linear and quadratic features to reduce possible overfitting risks, following approaches recently adopted in other studies (Brambilla et al., 2022). The selected number of iterations was 660, and the selected regularisation multiplier in the tuned model was 0.5. After selecting the regularisation multiplier, we removed the variables with null effect (Lambda equal to 0), and tuned the model selecting number of iterations, regularisation multiplier, variables to be included in the model, functions (linear and/or quadratic). The continuous suitability value was transformed into a value constrained between 0 and 1 by means of a cloglog transformation. To easy model interpretation, the suitability map (continuous values based on model predictions) was reclassified into discrete categories according to some commonly adopted cloglog thresholds. Predicted suitability values were hence reclassified into unsuitable (lower than the minimum training presence), low (between the minimum training presence and the 10th percentile training), average (between the 10th percentile training and maximum training sensitivity plus specificity), high suitability (higher than maximum training sensitivity plus specificity). To assess model reliability, we computed AUC (Area Under the Curve of the Receiver Operating Characteristics) and TSS (True Skill Statistic) over training and testing datasets. While the absolute value of TSS and AUC is sensitive to prevalence and extent (Lobo et al., 2008), similar values over spatially independent partitions provide an indication of model robustness. We also computed the omission rates on the test data considering the 10th percentile threshold on training data; values clearly higher than expected (0.1) would suggest overfitting (**Table S3**). To provide a further assessment of model validity and extrapolation potential, we checked whether the occurrence data available from additional areas (**Table S4**), which were not used for the modelling procedure, overlap with areas of predicted suitability. The procedure was implemented in R, using the packages dismo (Hijmans et al., 2017) and SDMtune (Vignali et al., 2020), while maps were produced with QGIS 3.18 (QGIS.org, 2021, available at: http://www.qgis.org).

3. Results and discussion

Laboratory investigations suggest that both *Ae. j. japonicus* and *Ae. koreicus* (Fig. 1) are competent vectors for the transmission of several arboviruses and filarial nematodes, despite a lack of field data about their actual role as active vectors for some pathogens (Ciocchetta et al., 2018; Miles, 1964; Montarsi et al., 2015). Knowing the geographical distribution of these invasive mosquitoes is thus very useful to identify areas in which surveillance campaigns should be done to limit the spread of these insects (Koban et al., 2019).

In our study, during 2021, a total of 109 breeding sites have been monitored to assess the distribution of *Ae. koreicus* and *Ae. j. japonicus* in Northern Italy (Figs. 2 and 3). A detailed description of the screened municipalities, within each district, and the collected samples, namely 7445 individuals, is given in **File S1**. Data focusing on *Ae. koreicus* and *Ae. j. japonicus* are reported in Table 1.

Ae. koreicus larvae were collected in several districts of this part of the country, i.e., Bergamo, Brescia, Como, Lecco, Sondrio and Alessandria, Asti, Biella, Turin, respectively for Lombardy and Piedmont

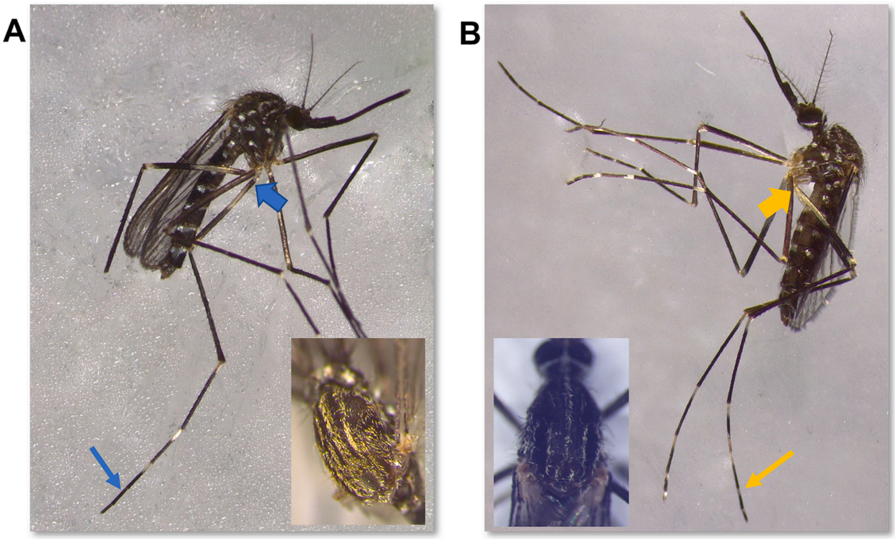

Fig. 1. Samples of adult female mosquitoes of *Aedes japonicus japonicus* and *Aedes koreicus*. *Ae.* j. *japonicus* adult mosquitoes (A) show a dark subbasal band on the white hind femur (thick blue arrow) and lack the pale band at the 4th hind tarsomere (blue thin arrow). On the contrary, *Ae. koreicus* (B) lacks the dark subbasal band (thick orange arrow) and has a white band on the 4th tarsomere (orange thin arrow). Mesonotum harbouring the two typical patterns of lines is shown for both species/sub-species.

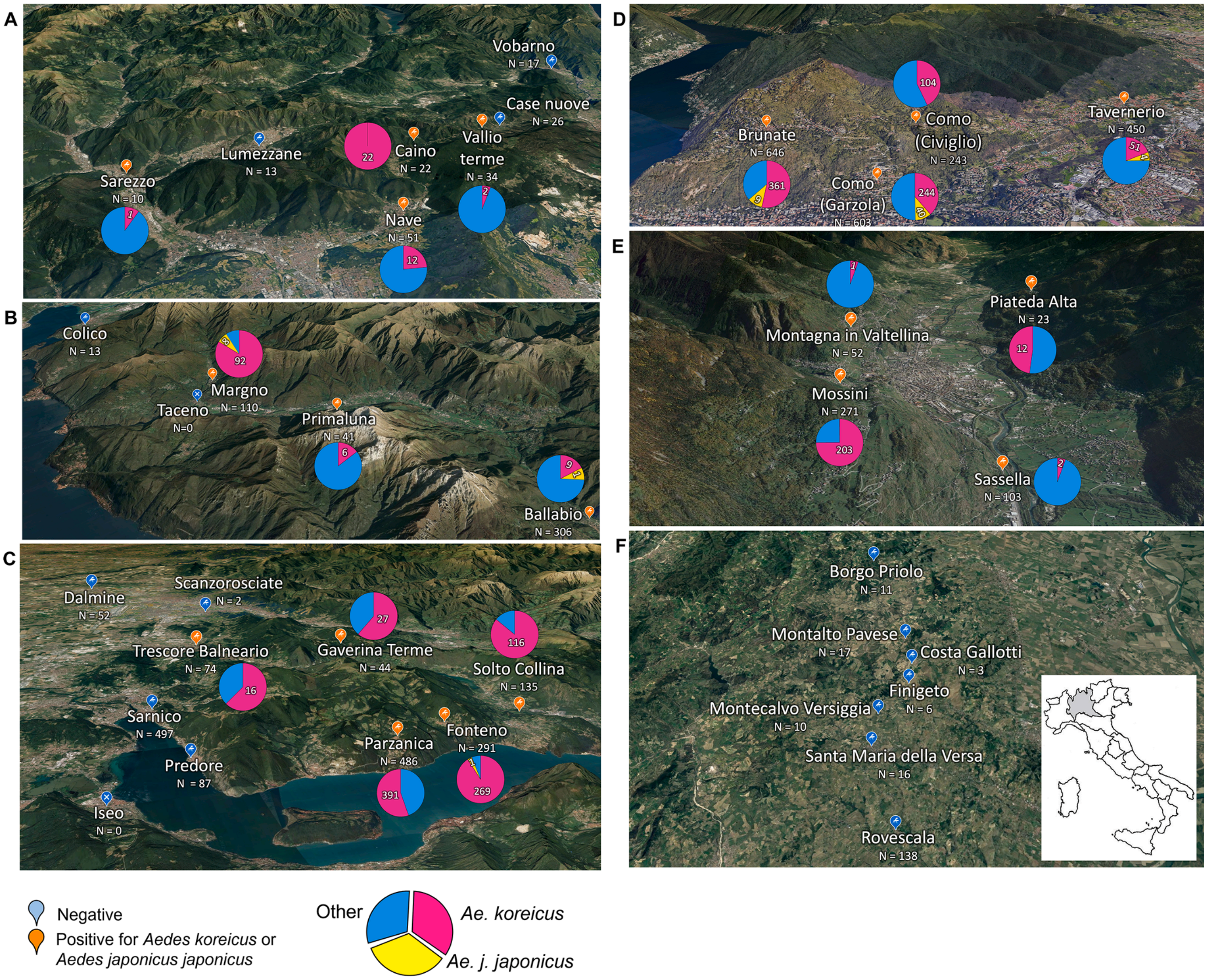

Fig. 2. Presence of *Aedes koreicus* and *Aedes japonicus japonicus* in Lombardy region. Surveys were carried out in the districts of Brescia (A), Lecco (B), Bergamo (C), Como (D), Sondrio (E), and Pavia (F) in the Lombardy region. The total number of collected larvae is given for each municipality. The number of *Aedes koreicus* and *Aedes j. japonicus* larvae is indicated in the pie chart in fuchsia and yellow slices, respectively.

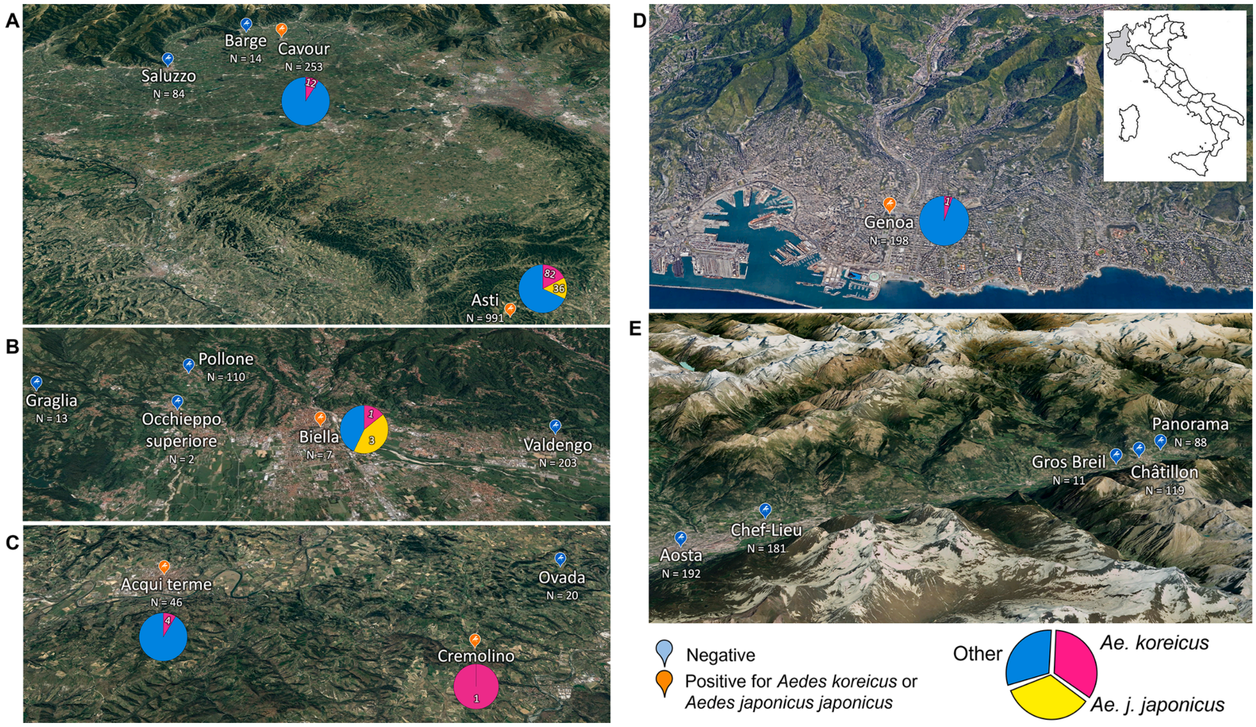

Fig. 3. Presence of *Aedes koreicus* and *Aedes japonicus japonicus* in Piedmont, Aosta Valley and Liguria regions. Surveys were carried out in the districts of Turin, Cuneo, Asti (A), Biella (B) and Alessandria (C) in the Piedmont region. Genova (D) and Aosta (E) provinces were screened in the Liguria and Aosta Valley regions, respectively. The total number of collected larvae is given for each municipality. The number of *Aedes koreicus* and *Aedes j. japonicus* larvae is indicated in the pie chart in fuchsia and yellow slices, respectively.

regions. The presence of *Ae. koreicus* was also confirmed in the Genoa district (Liguria region), while it was not detected in Aosta Valley.

In Lombardy, 55% of the 76 surveyed breeding sites were positive for *Ae. koreicus*. During June/July 2021, 68% of the 19 breeding sites surveyed in the province of Bergamo were positive for *Ae. koreicus*, while we found only one *Ae. j. japonicus* larva. Given the high prevalence of *Ae. koreicus,* we decided to monitor its presence also during late autumn (at the end of November) in two municipalities (Parzanica and Fonteno), finding the species in the majority of the sites. This observation suggests that *Ae. koreicus* remains active at least till the late autumn in this region, even if the total number of collected larvae was much lower than during the summer season.

A high prevalence of *Ae. koreicus* during summer was also detected in all the screened breeding sites (11) in the district of Como. Additionally, 36% of the breeding sites in municipalities in the province of Brescia, 50% in the province of Lecco and 50% in the province of Sondrio were found positive for this invasive mosquito during early autumn collections. Notably, this is the first report of the presence of *Ae. koreicus* in the districts of Lecco and Brescia: previous studies done in 2014 did not detect this invasive mosquito in this area (Montarsi et al., 2015).

Ae. j. japonicus was less distributed in our target sites and showed smaller populations than *Ae. koreicus*. Particularly, it was found in 7/11 breeding sites (64%) in the district of Como and 2/8 in the district of Lecco (25%), while in Brescia and Sondrio we did not find any *Ae. j. japonicus* larva. Even for this mosquito subspecies, this is the first report of its presence in this area (Table 1 and **S1**).

In Piedmont region, 43% of the 21 screened breeding sites resulted positive for *Ae. koreicus* presence and 14% for *Ae. j. japonicus*. *Ae. koreicus* was found in 4/4 breeding sites in Alessandria (100%), 1/7 in Biella (14%), 2/5 in the municipality of Cavour in the district of Turin (40%) and 2/3 in Mombarone in the district of Asti (67%). In the same region, we found *Ae. j. japonicus* in Biella (1/7 breeding sites, 14%) and in Mombarone (2/3 breeding sites, 67%) (Table 1 and **S1**).

In Genoa, in the region of Liguria, 1/3 breeding sites (33%) that we surveyed at the beginning of November was positive for *Ae. koreicus*. Finally, in the Aosta Valley region only *Cx. pipiens* s.l., *Cx. hortensis* and *Cs. longiareolata* larvae were collected, although we screened nine breeding sites in five different municipalities (Table 1 and **S1**).

We found evidence that *Ae. koreicus* have colonised a wider area in Northern Italy than *Ae. j. japonicus,* probably since it is better adapted to urban and peri-urban environments (Marcantonio et al., 2016). *Ae. j. japonicus* and *Ae. koreicus* are considered weak larval competitors in comparison to *Ae. albopictus*. Indeed, *Ae. j. japonicus* has a lower intrinsic capacity for population growth and survival than *Ae. albopictus*, and the latter develops faster than *Ae. koreicus* regardless of diet and density (Baldacchino et al., 2017). Nevertheless, *Ae. koreicus* and *Ae. j. japonicus* can establish earlier in spring (the former, generally one month before) and to remain active for a longer time in autumn, thus favouring their invasion of Europe even in presence of *Ae. albopictus* (Baldacchino et al., 2017; Cunze et al., 2016). For instance, the co-occurence of *Ae. j. japonicus* and *Ae. albopictus* was previously observed in countries such as Slovenia and in Northern Italy (Kalan et al., 2017). Noteworthy, the localities that we surveyed are not in the optimum range for the diffusion of *Ae. albopictus,* which is expected to peak during the summer at lower altitudes (the species has an optimum development temperature at 30°C) (Ballardini et al., 2019; Marini et al., 2019). During the summer season, indeed, *Ae. koreicus* was often predominant over the other mosquito species in the sampled area in a range of altitudes between 425 and 790 m a.s.l. (Bergamo and Como), while *Ae. albopictus* was predominant in Biella (between 275 and 737 m a.s.l.). In autumn, we found a higher amount of *Ae. koreicus* than *Ae. albopictus* larvae in Lombardy, in the Piedmont region in the district of Alessandria, while *Ae. albopictus* was predominant in Cuneo, Asti and Turin districts.

Given the large diffusion of *Ae. koreicus* in the sampled area, the occurrence data from this and previous monitoring activities (Gradoni et al., 2021) were used to generate a Maxent model for the prediction of habitat suitability for *Ae. koreicus* mosquitoes. The distribution model included the following nine variables (listed from highest to lowest

Table 1
Prevalence of *Aedes koreicus* and *Aedes j. japonicus* in the districts screened during the survey. The percentage of positive breeding sites and the prevalence of the invasive species on the total collected larvae is reported for each screened municipality. N (BS): number of the screened breeding sites, % BS_{K+}: percentage of breeding sites positive for *Ae. koreicus*, % BS_{J+}: percentage of breeding sites positive for *Ae. j. japonicus*, N (L): total number of collected larvae, % L_K: percentage of larvae of *Ae. koreicus*, % L_J: percentage of larvae of *Ae. j. japonicus*.

Region	District	Municipality	N (BS)	% BS_{K+}	% BS_{J+}	N (L)	% L_K	% L_J
Lombardy	Bergamo	Predore	1	0	0	87	0	0
Lombardy	Bergamo	Sarnico	1	0	0	497	0	0
Lombardy	Bergamo	Iseo	1	0	0	0	0	0
Lombardy	Bergamo	Trescore Balneario	3	66.67	0	74	21.62	0
Lombardy	Bergamo	Parzanica	12	75	0	486	80.45	0
Lombardy	Bergamo	Solto Collina	2	100	0	135	85.93	0
Lombardy	Bergamo	Fonteno	3	100	33.33	291	92.44	0.34
Lombardy	Bergamo	Gaverina Terme	2	100	0	44	61.36	0
Lombardy	Bergamo	Dalmine	1	0	0	52	0	0
Lombardy	Bergamo	Scanzorosciate	1	0	0	2	0	0
Lombardy	Bergamo	Total	27	66.67	3.70	1668	49.10	0.06
Lombardy	Brescia	Sarezzo	3	33.33	0	10	10	0
Lombardy	Brescia	Nave	3	33.33	0	51	23.53	0
Lombardy	Brescia	Caino	1	100	0	22	100	0
Lombardy	Brescia	Vallio Terme	1	100	0	34	5.88	0
Lombardy	Brescia	Case Nuove	1	0	0	26	0	0
Lombardy	Brescia	Vobarno	1	0	0	17	0	0
Lombardy	Brescia	Lumezzane	1	0	0	13	0	0
Lombardy	Brescia	Total	11	36.36	0	173	21.39	0
Lombardy	Como	Como	6	100	66.67	846	41.13	4.73
Lombardy	Como	Brunate	3	100	33.33	646	55.88	1.39
Lombardy	Como	Tavernerio	2	100	100	450	11.33	0.89
Lombardy	Como	Total	11	100	63.64	1942	39.13	2.73
Lombardy	Lecco	Ballabio	4	50	25	314	2.87	0.32
Lombardy	Lecco	Colico	1	0	0	13	0	0
Lombardy	Lecco	Margno	1	100	100	110	83.64	7.27
Lombardy	Lecco	Taceno	1	0	0	0	0	0
Lombardy	Lecco	Primaluna	1	100	0	41	14.63	0
Lombardy	Lecco	Total	8	50	25	478	22.38	1.88
Lombardy	Pavia	Rovescala	2	0	0	138	0	0
Lombardy	Pavia	Santa Maria della versa	1	0	0	16	0	0
Lombardy	Pavia	Montecalvo Versiggia	2	0	0	10	0	0
Lombardy	Pavia	Finigeto	1	0	0	6	0	0
Lombardy	Pavia	Costa Gallotti	1	0	0	3	0	0
Lombardy	Pavia	Montalto Pavese	1	0	0	17	0	0
Lombardy	Pavia	Borgo Priolo	1	0	0	11	0	0
Lombardy	Pavia	Total	9	0	0	201	0	0
Lombardy	Sondrio	Sassella	3	66.67	0	103	1.94	0
Lombardy	Sondrio	Mossini	2	50	0	271	74.91	0
Lombardy	Sondrio	Montagna in valtellina	3	33.33	0	52	1.92	0
Lombardy	Sondrio	Piateda Alta	2	50	0	23	52.17	0
Lombardy	Sondrio	Total	10	50	0	449	48.55	0
Piedmont	Alessandria	Acqui Terme	2	100	0	47	8.51	0
Piedmont	Alessandria	Cremolino	1	100	0	1	100	0
Piedmont	Alessandria	Ovada	1	100	0	20	25	0
Piedmont	Alessandria	Total	4	100	0	68	14.71	0
Piedmont	Asti	Mombarone	3	66.67	66.67	991	8.27	3.63
Piedmont	Biella	Pollone	3	0	0	110	0	0
Piedmont	Biella	Graglia (Santuario)	1	0	0	13	0	0
Piedmont	Biella	Occhieppo Superiore	1	0	0	2	0	0
Piedmont	Biella	Biella	1	100	100	7	14.29	42.86
Piedmont	Biella	Valdengo	1	0	0	203	0	0
Piedmont	Biella	Total	7	14.29	14.29	335	0.30	0.90
Piedmont	Cuneo	Saluzzo	1	0	0	84	0	0
Piedmont	Cuneo	Barge	1	0	0	14	0	0
Piedmont	Cuneo	Total	2	0	0	98	0	0
Piedmont	Torino	Cavour	5	40	0	253	4.74	0
Aosta	Aosta	Panorama	1	0	0	88	0	0
Aosta	Aosta	Châtillon	3	0	0	119	0	0
Aosta	Aosta	Gros Breil	1	0	0	11	0	0
Aosta	Aosta	Chef-Lieu	2	0	0	181	0	0
Aosta	Aosta	Aosta	2	0	0	192	0	0
Aosta	Aosta	Total	9	0	0	591	0	0
Liguria	Genoa	Genoa	3	33.33	0	198	0.51	0

permutation importance), comprising both LULC and climatic factors: urban areas, broadleaved forest, annual mean temperature, precipitation seasonality, coniferous forest, arable land, natural grassland, vineyards, pastures. The effect of the different predictors is displayed in **Fig. S1**, and the permutation importance is reported in **Table S2**. The resulting map of environmental suitability is shown in Fig. 4, with four levels of suitability (i.e., unsuitable, low, average, high suitability).

Based on the Maxent model, variables related to land use, primarily the degree of urbanisation and forest cover, were found to be the most relevant for the evaluation of habitat suitability, followed by climate-

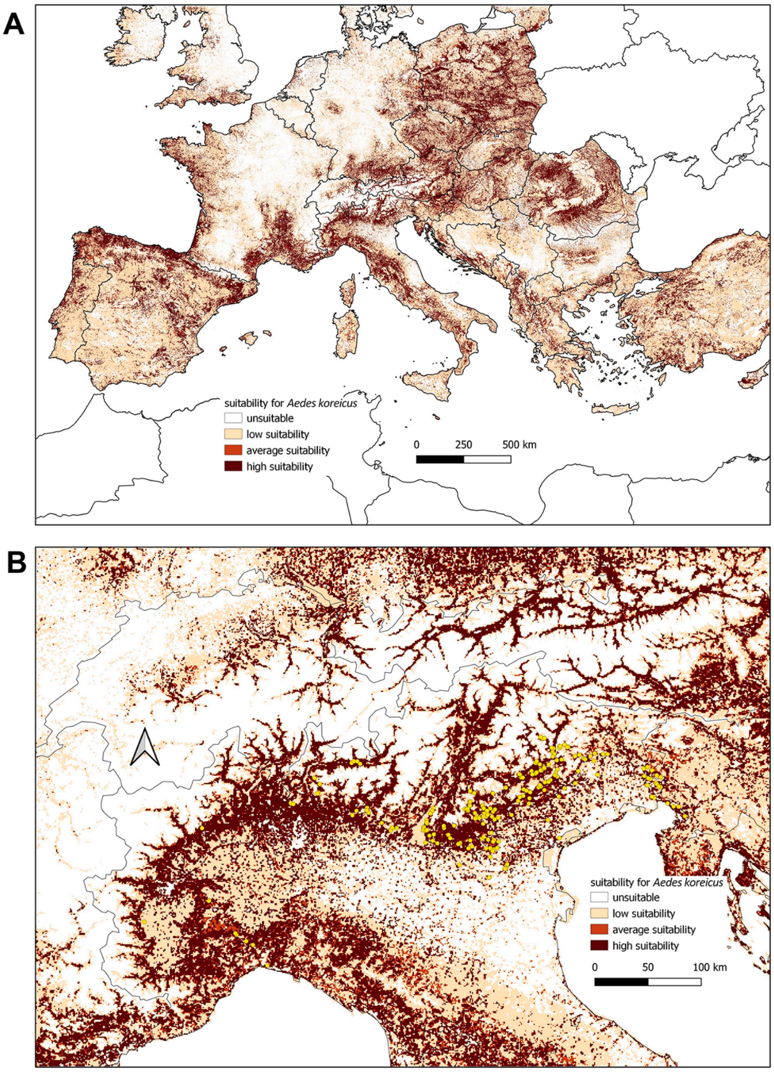

Fig. 4. Distribution of *Aedes koreicus* species predicted by the Maxent model for habitat suitability in Central and Southern Europe (A) and Northern Italy (B). Occurrence points used to generate the model are shown on the map as yellow circles.

related variables, such as mean annual temperature and precipitation seasonality. The presence of *Ae. koreicus* is favoured by an intermediate degree of urbanisation, which seems to be associated with the presence of mixed forests, i.e. broadleaved and coniferous forests (**Fig. S1**). Additional variables related to land use, i.e., the presence of arable land, natural grassland, vineyards and pastures, were also important for defining habitat suitability (**Table S2**). Vineyards and pastures were positively associated with the presence of *Ae. koreicus*, maybe because of the exploitation of vegetation for recovery or the availability of farming animals for the feeding (**Fig. S1**). On the contrary, areas largely cultivated or lacking forest vegetation, such as arable land and natural grassland, were negatively correlated with the presence of this invasive mosquito. Thus, peri-urban areas, i.e. peripheric urban areas that are in proximity with broadleaved and coniferous forests, vineyards and pastures, constitute a suitable environment for the establishment of this species. These findings reflect the features of the sites in which we collected larvae of this species. Indeed, positive breeding sites were in small municipalities surrounded by forests at different elevation. In this scenario, small and big water containers were individuated in private gardens or lands in which we observed the presence of pets or farmed animals, such as cats, dogs, chickens, rabbits, and horses, or in uncultivated lawns close to forests, where wild animals were probably present.

Annual average temperature showed a great permutation importance for the generation of the model. Highly suitable areas for the establishment of *Ae. koreicus* were characterised by mid-high annual average temperatures, i.e. between 10 and 15°C. This range is typical of sub-alpine areas overlooking the Po River valley and of territories surrounding the Apennine mountains, which reflects the distribution of highly suitable areas shown in the distribution maps generated through the Maxent model. Additionally, an intermediate precipitation seasonality, i.e. the coefficient of variation of precipitation throughout seasons, seems to be peculiar to highly suitable areas.

Extrapolating the model obtained with data from Northern Italy to Central and Southern Europe suggested the presence of highly suitable areas in countries where *Ae. koreicus* has not yet been reported, such as Southern and North-Eastern France, Western Portugal and North-Eastern Spain, or expansion areas in colonised countries, such as Italy. Alpine areas and along the Apennines, as well as major islands, where the species has not been reported so far, were denoted as highly suitable and may represent areas of future expansion.

The model was robust, performing well on both training and testing datasets (**Table S3**). Occurrence data derived from previous studies were used to validate model predictions (**Table S4,** see Supplementary Material and related references). Occurrence data from Hungary, Slovenia and Austria all fell into highly suitable areas. The same was the case for Swiss occurrence data, where the species was reported in correspondence or in proximity to highly suitable areas, and for Northern Italy (Trentino Alto-Adige region) (**Fig. S2**). On the contrary, in Belgium and in Germany, the species was reported in poorly suitable areas (**Fig. S3**). In these countries, *Ae. koreicus* seems to have a low rate of expansion, which could be caused by repeated colonisation of poorly suitable areas. Noteworthy, we were not able to recover precise coordinates for the majority of survey sites in Germany and high suitable areas are present in proximity to the coordinates inferred for collections in Monaco, Augusta and Wiesbaden municipalities.

However, caution should be adopted when looking at the predicted suitability outside the study area. In fact, even if the model seems robust and predictions outside the calibration area are largely consistent with available data from other regions, our study took place in an area where the species' distribution might still have to reach a full equilibrium with climate and landscape features. If true, the relationships between occurrence and environmental traits could provide a partial representation of the species' ecological niche. Nevertheless, the model's extrapolation ability over distant areas points towards a potentially reliable prediction of distribution at a broader scale.

The relevance of landscape (i.e. land use and other elements which can affect the presence of breeding sites, blood hosts and predators) has previously been evaluated in modelling the distribution of *Ae. j. japonicus* mosquitoes (Kerkow et al., 2019). Specifically, variables related to landscape structure appear to favour the presence of the species in climatically unsuitable regions (Kerkow et al., 2019). This may be in line with the high permutation importance of several land use-related variables in our model. Previous studies have also assessed the habitat suitability for *Ae. koreicus,* with forest coverage in adult trapping areas and vegetation variability, as the two key factors associated with the presence of this species (Baldacchino et al., 2017). Particularly, in a 2-year survey carried out in the Trentino region, adult mosquitoes of this species were predominant in the trapping site located in the forested area, being less abundant than *Cx. pipiens* in the urban one (Baldacchino et al., 2017). However, previous published models on *Ae. koreicus* suggested that the vegetation variability is less relevant than temperature (Marcantonio et al., 2016). Nevertheless, the dataset exploited for the generation of the models referred to a more limited territory of Northern Italy and vegetation variability was described on the basis of Normalised Difference Vegetation Index (NDVI). Moreover, the spatial resolution is different from that used in our study (1 km) and it can account for the different relevance of this environmental factor.

These studies suggested that the areas suitable for *Ae. koreicus* colonisation were located at low altitude (0–800 m a.s.l.), with a peak in suitability around 400-500 m a.s.l. (Marcantonio et al., 2016). Results from our survey are mostly in line with this statement, even if, based on our model, altitude is not a relevant variable for the evaluation of habitat suitability. Breeding sites for *Ae. koreicus* larvae were in a wide altitudinal range and the peak in suitability that was previously reported might be a spurious correlation, given the abundance of peri-urban areas at specific elevations in the survey region.

Temperature was reported as another factor playing a major role in determining the habitat suitability for the establishment of *Ae. koreicus* (Marcantonio et al., 2016). It was observed a monotonic increase of presence probability in a range of maximum temperature of the warmest month between 22 and 28°C and an asymptotic trend at warmer values. Additionally, extreme temperature seasonality was considered unsuitable (Marcantonio et al., 2016). Our laboratory observations and a published study (Marini et al., 2019) confirm this trend; in fact, temperatures above 28°C are not optimal for the development of pupae and adults, which prefer temperate conditions ranging from 20–26°C. On the contrary, larvae show a similar survival rate between 13°C and 33°C (Marini et al., 2019). This observation agrees with the fact that this mosquito species seems to be able to better tolerate cold climates than *Ae. albopictus* (Marcantonio et al., 2016). Therefore, we can expect a wider temporal and geographical range to be colonised by *Aedes* mosquitoes in temperate areas, with a potential spread of *Aedes*-borne pathogens (Marcantonio et al., 2016).

4. Conclusion

Ae. koreicus has rapidly expanded its areal in Northern Italy likely from one or a few initial introduction sites, and it might further spread in the absence of control measures. Indeed, we found *Ae. koreicus* in districts that were negative for this species in previous screenings in the Lombardy region. The SDM generated from the occurrence data for *Ae. koreicus* in Northern Italy predicts further highly suitable areas in our country and in Europe. For this reason, investigations on the main modes and routes of dispersal of this invasive species and an increase of monitoring efforts should be prioritized.

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CRediT authorship contribution statement

Irene Arnoldi: Investigation, Data curation, Writing – original draft, Visualization. **Agata Negri:** Investigation, Data curation, Writing – original draft. **Laura Soresinetti:** Investigation, Data curation, Writing – original draft. **Mattia Brambilla:** Methodology, Software, Validation, Formal analysis. **Davide Carraretto:** Investigation, Visualization. **Fabrizio Montarsi:** Resources. **Paolo Roberto:** Resources. **Andrea Mosca:** Resources. **Diego Rubolini:** Methodology. **Claudio Bandi:** Supervision, Funding acquisition. **Sara Epis:** Conceptualization, Investigation, Resources, Writing – original draft, Project administration. **Paolo Gabrieli:** Conceptualization, Investigation, Resources, Writing – original draft, Project administration, Funding acquisition.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.actatropica.2022.106536.

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