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Evidence of expanding range and environmental suitability for *Culex perexiguus* in Northern Italy (Emilia-Romagna, 2025)

Mattia Calzolari · Annalisa Grisendi · Mara Scremin · Gastone Dalmonte · Emanuele Callegari · Anna Beatrice Casano · Francesco Severini · Gabriele Antolini · Alessandro Albieri · Dottori Michele

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Abstract

Culex perexiguus, a mosquito typically associated with hot and semi-arid environments across North Africa, the Middle East, and southwestern Asia, has recently emerged as a potential public health concern in parts of southern Europe due to its demonstrated vector competence for multiple arboviruses, particularly West Nile virus (WNV). In 2025, 84 specimens of this mosquito were collected across 22 sites in Emilia-Romagna, northern Italy, within the regional WNV entomological surveillance program; DNA barcoding of 21 individuals confirmed morphological identification. Comparison with entomological data from 2009 revealed a concurrent decrease of another mosquito, *Culex modestus*, suggesting a possible northward shift in suitable areas for both species. Ecological niche modelling based on presence records of *Cx. perexiguus* indicated an estimated distribution primarily linked to the availability of suitable breeding habitats, and the meteorological anomalies recorded in 2025 (compared to 2001–2020) likely favoured its presence. The unexpected expansion of *Cx. perexiguus* into Emilia-Romagna, in an area characterised by a temperate continental climate, underscores the broader changes in mosquito distribution potentially linked to climate change. Its presence may have significant implications for public health, vector surveillance, and control strategies, and will therefore require careful monitoring in the coming years.

Introduction

The mosquito *Culex perexiguus* Theobald, 1903, belongs to the Pipiens group and to the Univittatus subgroup, which also includes the morphologically similar species *Culex (Culex) univittatus* Theobald, 1901, *Culex neavei* Theobald, 1906 and *Culex fuscocephala* Theobald, 1907[1]. Although some of these taxa were historically synonymised with *Cx. univittatus*, morphological and reproductive studies have confirmed their taxonomic distinctiveness, revealing significant genetic incompatibilities among them[2,3,4,5].

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Culex perexiguus exhibits a broad circum-Mediterranean Middle Eastern distribution, encompassing southern Europe, North Africa, and southwestern Asia up to India and Myanmar[6,7]. In Europe this mosquito is well established in Portugal and Southern Spain and also reported in Greece, Bulgaria, Albania, Malta, Italy[8]. The past synonymization with *Cx. univittatus* has generated ambiguity in reports of *Cx. perexiguus*, which is considered the species of the subgroup occurring in Europe[9]. Nevertheless, *Cx. univittatus* has also been reported from the Iberian Peninsula[10].

Culex perexiguus is predominantly associated with natural, but also anthropogenic, aquatic habitats such as wetlands, marshes, road puddles, stream pools, ponds, wells, ditches, irrigation channels and rice paddies[6,11,12,13,14]. This mosquito prefers larger water bodies, especially in arid areas, with emergent vegetation, clean to moderately polluted water and tolerates some degree of salinity[5,6].

Although *Cx. perexiguus* is not typically associated to artificial containers, it may occur in drums, wells, artificial basin, cement/stone manufacts[6,12,15], and may breed in peri-urban areas[16,17]. Seasonal dynamic of this mosquito is characterized by an increased abundance in late summer and autumn[6,11].

Host preference studies indicate a strong ornithophilic tendency of *Cx. perexiguus*, with wild birds serving as primary bloodmeal sources, but it occasionally feeds on mammals, including humans and horses[18,19]; this mosquito can bite in-door at night[12].

These feeding preferences amplify the epidemiological importance of *Cx. perexiguus* as West Nile virus (WNV) vector, particularly in Mediterranean areas. Indeed, *Cx. perexiguus* is recorded as main WNV vectors in Israel[20,21] and emerged as dominant WNV vector in southern Spain[22,23]; WNV was isolated from field collected pools in Egypt[24] and detected by biomolecular methods in Algeria[25], Tunisia[26] and Turkey[27]. Moreover, this mosquito is considered a very relevant vector of Sindbis virus, found positive to this virus in the field in Egypt[24], Algeria[28] and Spain[29], and was incriminated as competent vector of Rift valley fever virus in Egypt[30].

Following the detection of WNV circulation and the subsequent reporting of human cases of neuroinvasive disease in Emilia-Romagna in 2008[31,32], the virus has become an increasingly relevant public health concern in Italy. The primary WNV vector in northern Italy is *Culex pipiens* Linnaeus, 1758, which, due to its wide distribution and high abundance, is among the most common mosquito species in the region[33]. Another Palaearctic species involved in WNV transmission is *Culex modestus* Ficalbi, 1889, which exhibits high vector competence[34] but is associated with specific habitats such as wetlands and renatured areas, and is therefore less widespread than *Cx. pipiens*[11,35].

As part of integrated West Nile virus surveillance, an entomological plan, based on attractive traps, was active until 2008 in Emilia-Romagna, northern Italy. Since 2010, traps have covered all lowland areas of the region, with the plan designed primarily to maximize the collection of *Cx. pipiens*, although other *Culex* mosquitoes, particularly *Cx. modestus*, have also been captured in smaller numbers. The unexpected sampling of several *Cx. perexiguus* specimens in different sites surveyed in this plan in 2025 is reported in this work.

Results

Collection of *Culex mosquitoes* (2009–2025)

Almost four million of *Culex* mosquitoes were collected in the frame of the entomological WNV surveillance in the period from 2009 to 2025, s from about 130,000 in 2017 to more than 400,000 in 2023, in a number of traps ranging from 64 to 109 per season (Fig. 1a, Table S1). The more abundantly collected *Culex* species was *Cx. pipiens* followed by *Cx. modestus*, even with substantially lower numbers, while the mosquito *Cx. perexiguus* was collected only in 2025(Fig. 1b, c, d).

The seasonal dynamics of *Cx. pipiens* showed a peak at the end of June, while *Cx. modestus* was more abundant at the end of the season (end of September) (Fig. 1b). The seasonal abundance of *Cx. pipiens*, exemplified

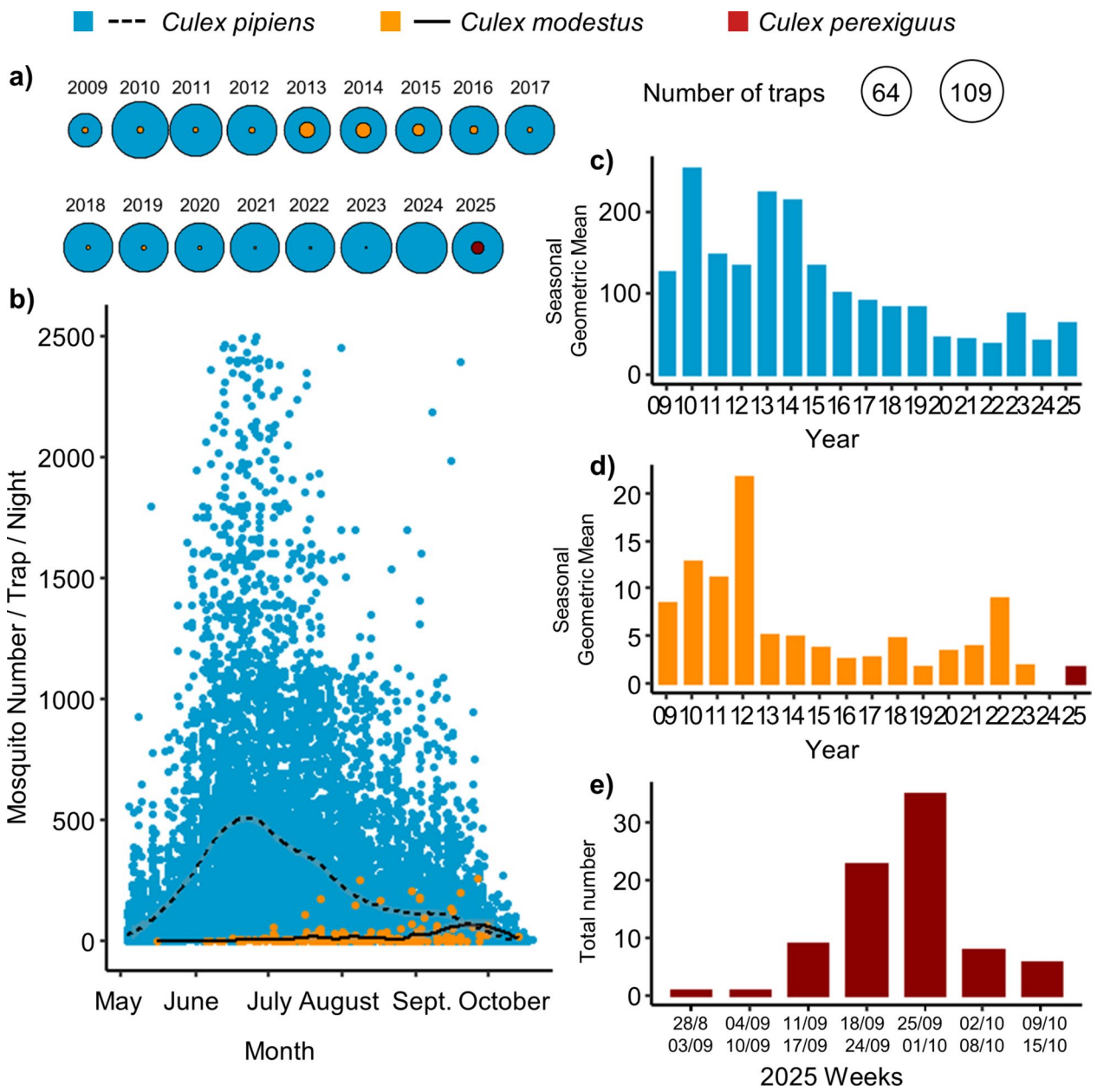


Fig. 1 Entomological data referring to *Cx. pipiens*, *Cx. modestus*, and *Cx. perexiguus*, collected within the framework of the Emilia-Romagna WNV surveillance program, 2009–2025. (**a**) Number of entomological traps operating during the different seasons, with indication of those in which the different mosquito species were captured (*Cx. pipiens* was sampled in all traps every year). (**b**) Seasonal dynamics of *Cx. pipiens* and *Cx. modestus*, represented by the interpolation line of all samplings performed during the study period. (**c**–**d**) Abundance of *Cx. pipiens*, *Cx. modestus*, and *Cx. perexiguus*, represented by the geometric mean of mosquitoes collected during the different years. (**e**) Total number of *Cx. perexiguus* mosquitoes collected on a weekly basis in 2025.

by the geometric mean was lower in the later years of the study than in the earlier years; however, this species was collected at all monitored sites in all seasons (Fig. 1a 1c, Table S1). The mosquito *Cx. modestus* had become less abundant over time, culminating in absence of collection in 2024 and 2025 (Fig. 1d) with a reduction also in terms of sites in which the mosquito was sampled (Fig. 1a).

In September 2025, a female mosquito bearing the *Cx. perexiguus* morphological characters was collected in a site in Imola municipality, Bologna province (Fig. 2, Table S2). *Cx. perexiguus* can be distinguished from

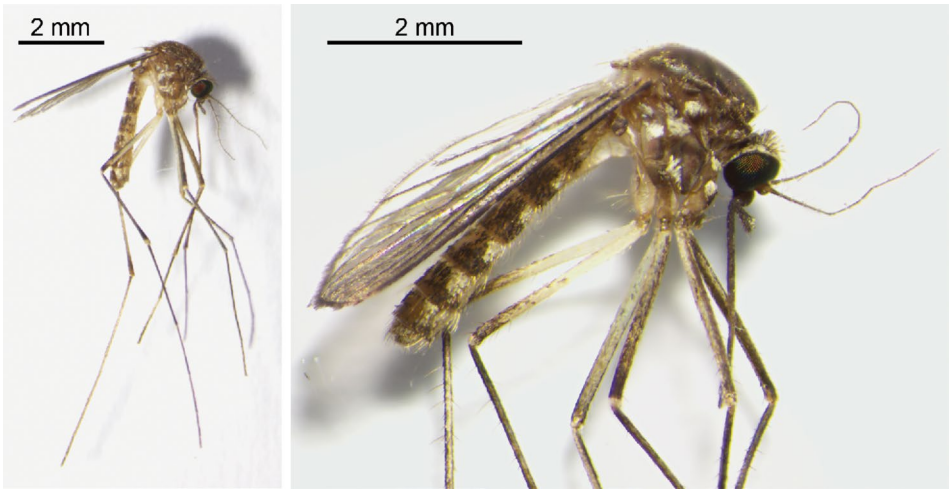


Fig. 2 Picture of *Cx. perexiguus*, entire specimen on the left and enlarged view of the thorax on the right (specimens collected on 16/09/2025 respectively at Molinella and Budrio municipalities, Bologna province).

Cx. pipiens, the most morphologically similar species collected in the surveyed area, by several morphological characters. The smaller body size, the presence of a patch of pale scales in the post-spiracular area[6,7], and a pale longitudinal stripe on the ventral surface of the hind tibia[11] are among the most distinct features separating the two species. In addition, *Cx. perexiguus* bears a white line at the base of the costal vein[12] and basal bands on the abdominal terga that are generally narrower than those of *Cx. pipiens* (Fisures S1, S2). The ring of pale scales on the femora and tibiae, sometimes reported as a distinctive character of *Cx. perexiguus*, may also occur in some melanic specimens of *Cx. pipiens* (F. Severine, personal communication) and should therefore be interpreted with caution. Overall, the two species have a similar general habitus, with a dark proboscis bearing light scales and the remainder of the body covered with golden and brown scales. Morphological differentiation between *Cx. perexiguus* and *Cx. univittatus* is more challenging and can be achieved by examining the length of the pale stripe on the hind tibia and the morphology of the male genitalia[10].

Following this first finding, 84 *Cx. perexiguus* females were collected in 22 sites in 6 provinces (Fig. 3), the site with the maximum number of specimens collected (33 in four different samplings) was the same as the first record. The last *Cx. perexiguus* specimen was collected on October 14, 2025, which was also the last day of WNV entomological surveillance (Fig. 4).

Barcoding identification

Identification of 21 mosquitoes, collected across 20 sites, was confirmed by sequencing the Cytochrome c oxidase subunit I (COI) gene. The obtained sequences were highly similar, with an overall mean p-distance of 0.014 (ranging from complete identity to a maximum of 0.062). These sequences were aligned with COI sequences of *Cx. perexiguus* and other members of the Univittatus subgroup (*Cx. univittatus*, *Cx neavei* and *Cx. fuscocephala*) retrieved from the BOLD database, resulting in an alignment of 266 sequences. In the maximum-likelihood phylogenetic tree, the sequences generated in this study clustered within a well-supported clade (bootstrap 98%) together with *Cx. perexiguus* sequences originating mainly from Africa, but also from Europe, particularly Spain, Italy, and Greece (Fig. 3, Figure S3). Notably, some sequences from South Africa, where this species is not expected to occur, also fell within the *Cx. perexiguus* clade, as previously reported[36]. Interestingly, sequences deposited as *Cx. univittatus* and *Cx. neavei* formed multiple clusters, whereas sequences of both *Cx. perexiguus* and *Cx. fuscocephala* formed two well-supported monophyletic clades (Fig. 3).

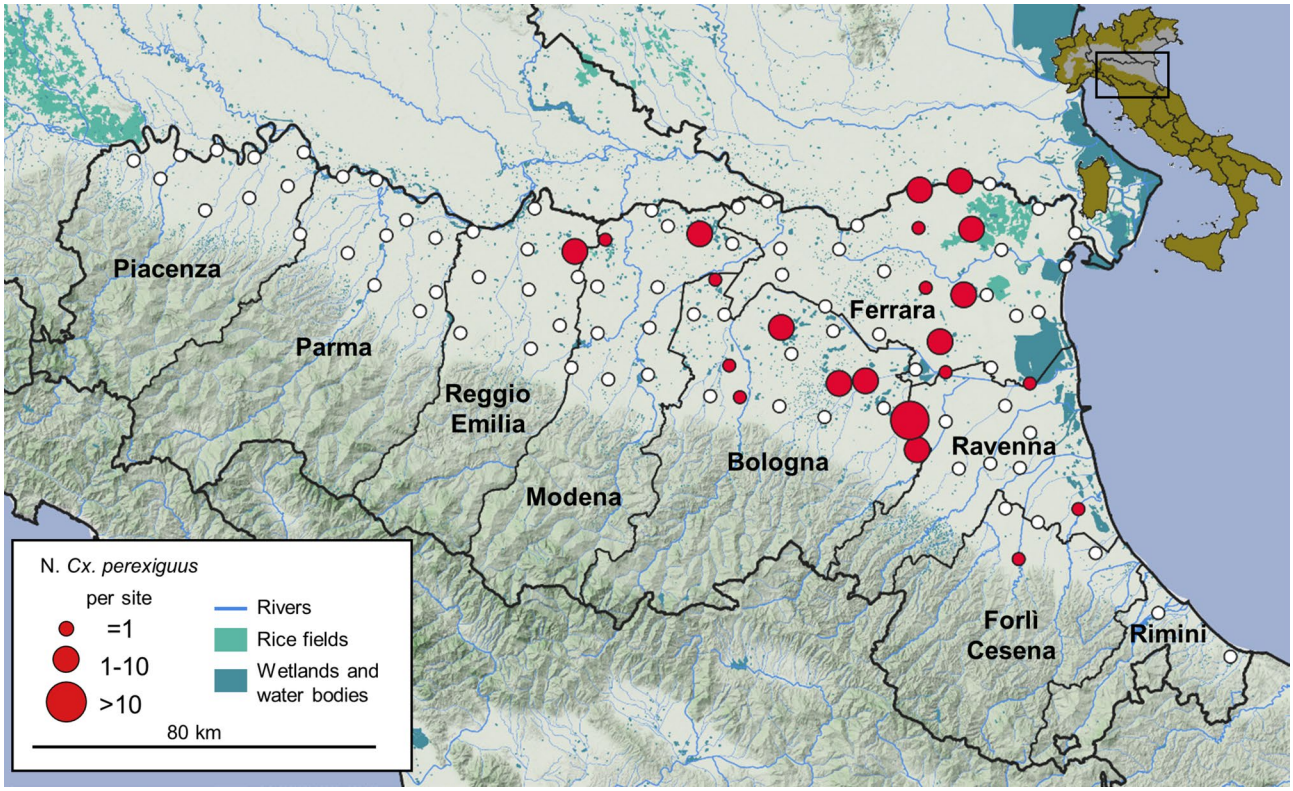


Fig. 3 Map of sites surveyed during the arboviral Emilia-Romagna plan in 2025, site in which *Cx. perexiguus* are collected highlighted in red, circles are proportional to the total number of collected specimens. Location of the study area on the map of Italy, with the Po Valley depicted in grey. The map was generated using QGIS v.4.2 (https://www.qgis.org).

Environmental suitability for *Cx. perexiguus*

The environmental suitability in the lowland area of Emilia-Romagna for *Cx. perexiguus* was summarized in an Ecological Niche Model (ENM) utilizing a set of 54 covariates (Table S3). Overall performance for the ENMs was good, with Area Under Curve (AUC) values of 0.765 (SD 0.098). The suitability map obtained by Maxent, revealed the highest host suitability for *Cx. perexiguus* in the eastern part of the region (Fig. 5). Variables related to potential breeding sites of this mosquito account for the highest relative contribution to the model (73.5%): USDA soil (35%) and the Corine Land Cover, (CLC) classifications (12.2%), proximity of wetland (15.5%) and proximity to water bodies < 1 km2 (10.8%) (Table S4). In categorical covariates, Aquents was the soil type contributing more to the model, between CLC classification the more relevant categories were inland marshes, followed by industrial and commercial areas (Figure S3). Other 23 covariates also contributed to the ENM, but each accounted for less than 5% (Table S4).

Meteorological conditions recorded in 2025, exemplified by the 13 bioclimatic indexes and recorded anomalies, appeared to have a limited influence on the ecological niche model ENM (Table S4). In fact, the differences in meteorological data (monthly temperatures and precipitation) between sites where the mosquito was detected and those where it was absent were minimal and smaller than the variability observed within each group (Figure S4). The surveyed area was relatively homogeneous with respect to these variables, and no association with the occurrence of *Cx. perexiguus* was detected. However, notable temperature anomalies relative to the 2001–2020 baseline were recorded in 2025 at sites where the mosquito was collected. In particular, June exhibited a positive anomaly of approximately + 3 °C in maximum, minimum, and mean temperatures (Fig. 6a). Additional thermal anomalies were observed in January, February, and March, especially in minimum temperatures, with increases

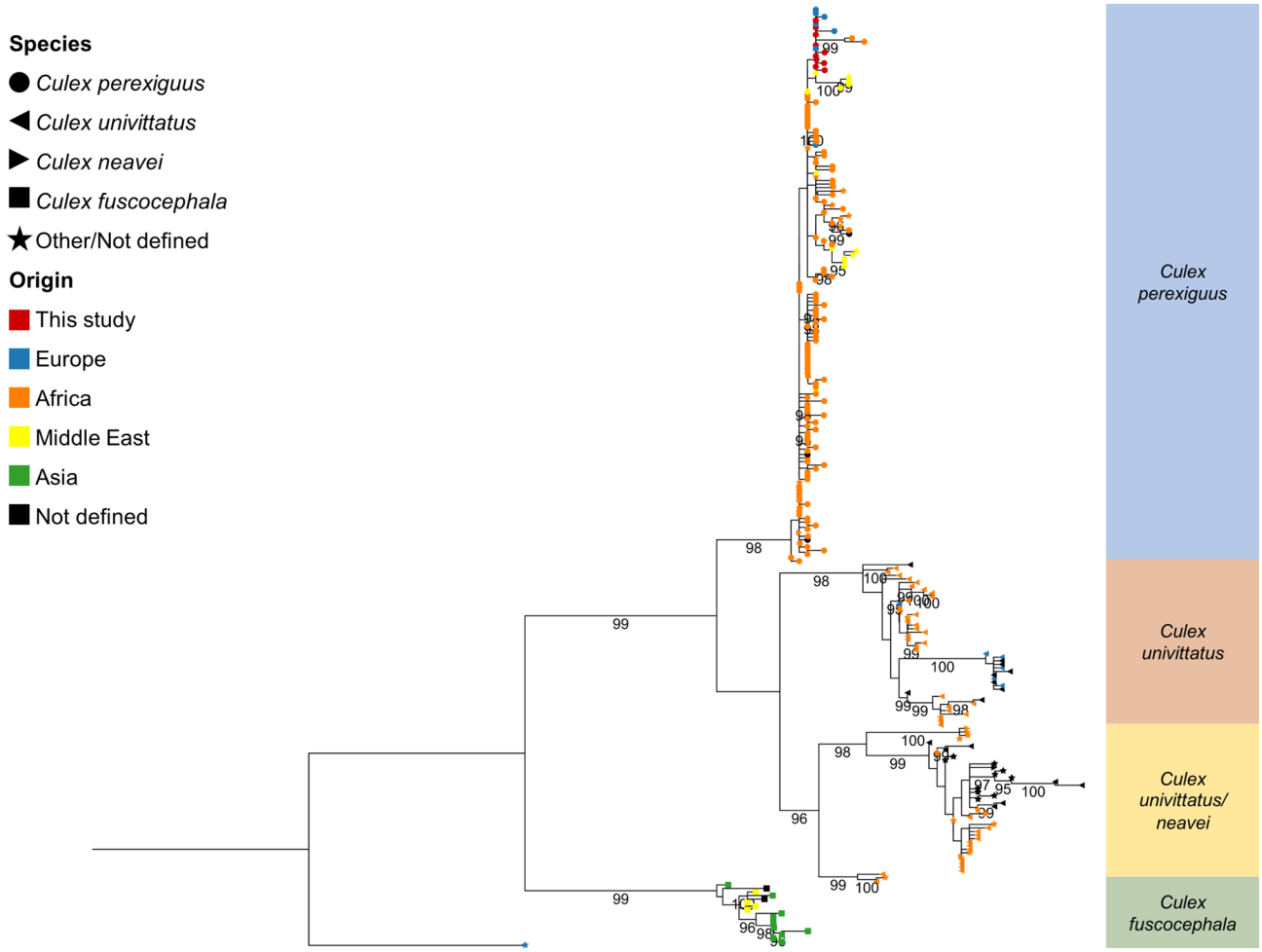


Fig. 4 Phylogenetic tree obtained by sequences from this work and BOLD sequences of the Univittatus subgroup. Sequence obtained in this work highlighted in red, leaf symbol according to the species name deposited in BOLD database and their geographical origin; reference rectangles indicate the Univittatus subgroup species present in the respective clades. *Cx. pipiens* sequence was used as the outgroup. Only bootstrap over 95% are shown near the branches.

ranging between +2 °C and +3 °C; conditions that may have been favourable for this species (Fig. 6a). Conversely, no clear patterns of precipitation anomalies were observed (Fig. 6b).

Discussion

As already mentioned, *Cx. perexiguus* is an Afro-Eurasian mosquito distributed across northern and sub-Saharan Africa, the Mediterranean basin, the Middle East, and extending into South Asia. This mosquito shows a fragmented distribution, with occurrence records localized in specific areas, as observed in the Iberian Peninsula, where the species is abundant in the southwestern regions but absent at comparable latitudes in the eastern part[14]. This mosquito, together with the nominal form of the subgroup, which has also been reported in Europe, is regarded as a species typical of the southern Mediterranean regions of Europe[37]. In Italy reports of *Cx. perexiguus* taxon are rare[9] and this mosquito has been recently signalled in Sardinia[38]. Old records of *Cx. univittatus*, dating back to the period of their synonymization, may actually refer to *Cx. perexiguus*[39] and are limited to southern Italy and Sicily[40,41]. These areas are however marginal in the overall distribution of the species, which is topically more common in the arid and semi-arid areas of North Africa and the Middle East[42].

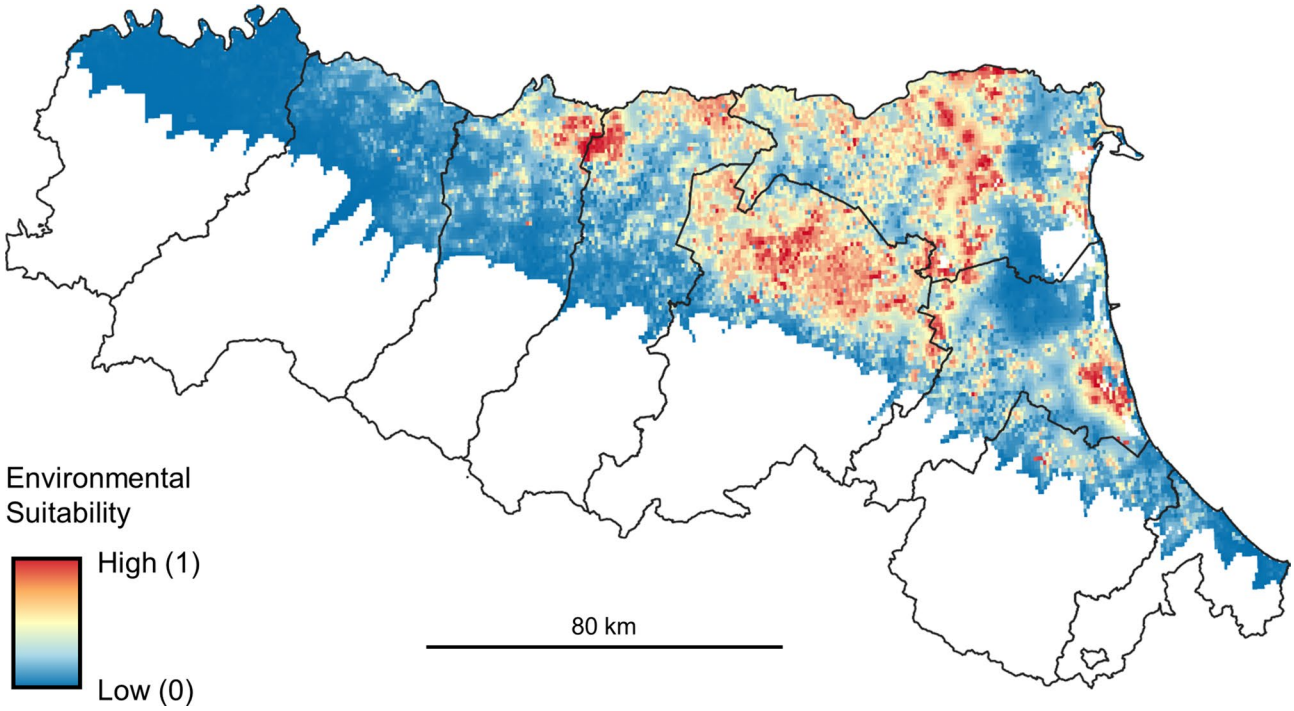


Fig. 5 Predicted probability of suitable conditions for *Cx. perexiguus* obtained by Maxent model in the study area, the lowland of Emilia-Romagna in 2025 (high suitability in red, low suitability in blue). The map was generated using QGIS v.4.2 (https://www.qgis.org).

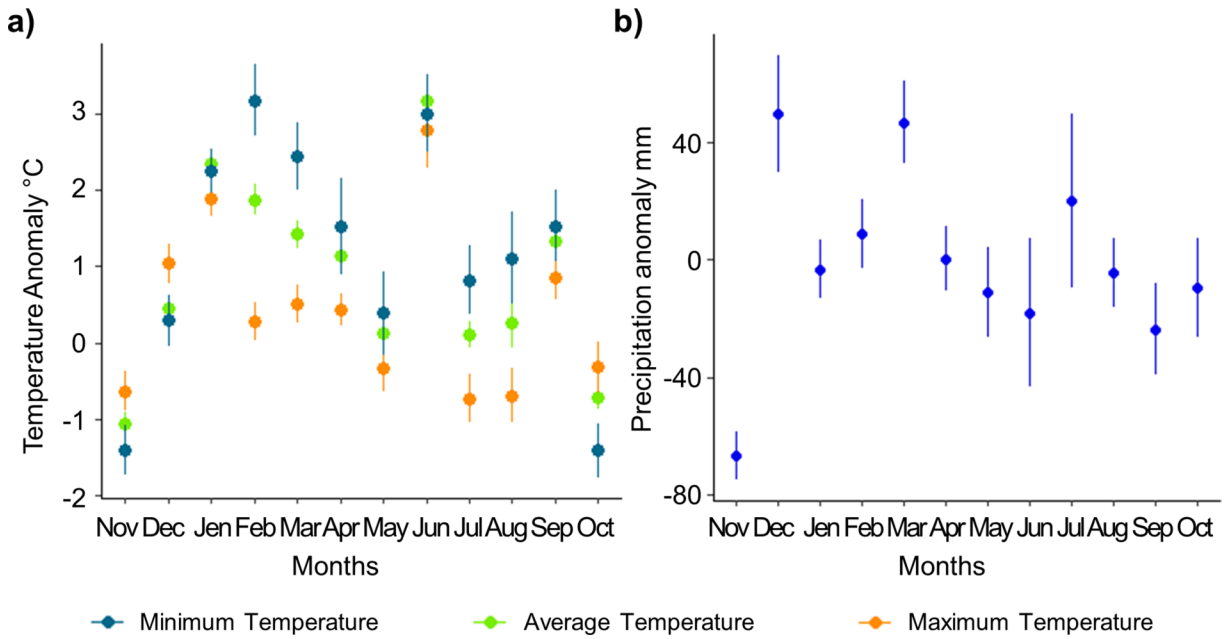


Fig. 6 Temperature (**a**) and precipitation (**b**) anomalies recorded from November 2024 to October 2025, relative to the 2001–2020 reference period, at sites where *Cx. perexiguus* was detected. Vertical bars represent the standard deviation.

The phylogenetic analysis of the sequences obtained supports the morphological identification of *Culex perexiguus* in the study area, as all generated sequences clustered within a well-supported clade together with reference sequences attributed to this species from Africa, the Middle East, and other regions of Europe. However, the tree obtained by sequences of the Univittatus subgroup reveals a degree of uncertainty, reflected by the occurrence of multiple clades associated with *Cx. univittatus* and *Cx. neavei*, and a possible broader distribution of *Cx. perexiguus* than previously reported. These findings suggest that the taxonomic relationships within the Univittatus subgroup could be partially unresolved and highlight the need for a more detailed characterization of its taxonomic relationships and the geographic distribution.

The report of *Cx. perexiguus* in the Po Valley, a region well outside to its previously described distribution, represents an unexpected finding. The collection of multiple specimens across 22 sites over a broad area of the region, may indicate a northward expansion of this mosquito.

The geographic expansion of *Culex* mosquitoes beyond their native ranges is an increasingly relevant global phenomenon, with climate change frequently recognised as a major driver. Since the early 2000s, at least twelve mosquito species native to Central and South America have been repeatedly reported in Florida, progressively establishing populations after colonizing the peninsula from overseas or western neighbouring regions, between these five belonging to the *Culex* genus[43]. Notably, one of these mosquitoes, *Culex coronator* Dyar and Knab, 1906, expanded poleward in both hemispheres, with established populations documented in the southern United States[44] and additional records extending as far south as Patagonia[45]. Similarly, the palearctic mosquito, *Culex tritaeniorhynchus* Giles, 1901, a primary vector of Japanese encephalitis virus, has expanded from its native range in Asia and the Middle East into parts of southern Europe[46] Africa[47] and Australia[48].

The comprehensive entomological surveillance system established in Emilia-Romagna enabled the detection of *Cx. perexiguus*, underscoring the added value of the regional framework beyond arbovirus monitoring. The detection of the species at the conclusion of the surveillance period aligns with its known tardive seasonal dynamic. It remains possible that this mosquito had been present at low densities for some time before its detection. However, as *Cx. perexiguus* was first identified only in 2025 within the West Nile virus surveillance program—an activity characterized by a standardized and seasonally consistent sampling effort since 2009—a recent introduction of the mosquito is probable.

Given that *Cx. perexiguus* preferentially inhabits natural environments, does not produce long-lasting eggs, and typically feeds on birds, introduction via commercial goods or passive transport by vehicles appears unlikely. Based on these observations, introduction pathways other than human-mediated transport are more consistent with the observed distribution of *Cx. perexiguus*. One possibility is a gradual range expansion from nearby areas, although this species has not been reported in adjacent regions. Another potential mechanism is passive dispersal by high-altitude winds, a process previously suggested for the spread of *Cx. tritaeniorhynchus* carrying Japanese encephalitis virus in Australia[49]. Notably, *Cx. perexiguus*, including WNV-positive individuals, has been detected in high-altitude windborne samples in Africa, supporting this potential mode of dispersal[50].

The environmental suitability model indicated that the presence of the mosquito is primarily associated with the availability of suitable larval habitats within the area. Specifically, it is linked to the presence and proximity of wetlands and inland water bodies, and, notably, to the Aquent soils. Aquents are soils formed from mineral sediments in persistently water-saturated environments, these characteristics strongly constrain their suitability for agricultural or infrastructural use, these soils are often occupied by wetland[51], and are then suitable for hosting breeding sites for this mosquito. Notably, this type of soils also proved to be a key factor in characterizing distribution of other mosquitoes of *Aedes* and *Anopheles* genus in the study area, highlighting the propensity of the terrain to host temporary water pools[52,53].

The relevant number of specimens collected in Emilia-Romagna in 2025 further suggests that environmental conditions during that season were favourable for this mosquito. Although the 2025 meteorological pattern does not appear to have influenced the presence of *Cx. perexiguus* in any specific portion of the surveyed area, significant temperature anomalies were recorded over the season. Considering that the population of this mosquito typically peaks in late summer and early autumn, the pronounced temperature anomaly recorded in June, may

have favoured the presence of this species. Other recorded pronounced anomalies were the increase in minimum temperatures of winter months, that may have contributed to the mosquito persistence. Increasing in winter minimum temperatures have been identified as key drivers of the northward expansion of *Culex quinquefasciatus* Say, 1823, and of the enlargement of its hybridization zone with *Cx. pipiens* in the United States, as the former species does not undergo true diapause but instead exhibits facultative quiescence in response to adverse environmental conditions[54]. Direct evidence of diapause physiology in *Cx. perexiguus* is lacking; however, the absence of a true winter diapause is a characteristic feature of thermophilic Mediterranean and Afrotropical mosquito species[55]. Such a physiological trait may have enhanced the establishment of *Cx. perexiguus* in new areas under scenarios of increased winter minimum temperatures.

Interestingly, the entomological data recorded in the surveyed area from 2009 showed a progressive decline of another species *Culex modestus*, a mosquito, which exploits larval habitats similar to *Cx. perexiguus*, but is typically more widespread in palearctic temperate regions[56]. In contrast, in the northern part of its range, *Cx. modestus* has shown evidence of geographic expansion, with an increased distribution reported in Bohemia between 2004 and 2007[57], followed by the first records in England in 2010[58] and Belgium in 2018[59].

Taken together, these findings indicate a likely northward shift of the suitable areas of the two species, with a gradual reduction of *Cx. modestus* in the study area and the occurrence and potential establishment of *Cx. perexiguus*. Climate warming has been proposed as a potential driver of distributional changes in mosquito species, through an increase in the suitability of northern areas for colonization[56]. As reported above, these observations are consistent with a broader phenomenon of poleward shifts reported for several mosquito species, indicating ongoing changes in their geographical ranges associated with climate change. If this phenomenon is confirmed, changes in mosquito fauna in a given area could result in new public health risk scenarios that will require careful monitoring and characterization to support appropriate public health actions.

A limitation of this study is that *Cx. perexiguus* was collected only in 2025. Nevertheless, the presence of this mosquito in the Emilia-Romagna region is a surprising observation, and the coming seasons will be crucial to determine whether this was an occasional occurrence or the beginning of a stable northward expansion. This mosquito is a highly competent vector of WNV; however, its transmission potential is primarily expressed in areas where it reaches significant population densities. Given the low abundance recorded in the sampled sites and the apparent association of this mosquito with natural and semi-natural breeding habitats, it does not yet appear to play a major epidemiological role in WNV transmission in the surveyed area.

However, *Cx. perexiguus* shows notable ecological plasticity, including the ability to colonize peri-urban habitats, although this behaviour has been documented only in restricted parts of its distribution range. The identification of industrial and commercial zones as a relevant predictor in the ENM may further support this potential.

Moreover, its seasonal population peak occurs later than that of the main WNV vector in the reference area, *Cx. pipiens*, potentially extending the temporal window of WNV transmission. Given these characteristics, any future increase in the abundance of *Cx. perexiguus* should be carefully monitored to mitigate the risks associated with its emergence and to anticipate future changes in arbovirus transmission dynamics.

Methods

Study area

The surveillance area, where WNV monitoring is active, corresponds to the Emilian part of the Po Valley, the largest plain in Italy. It covers approximately 10,000 km², is densely populated, and is characterized by intensive agriculture, rice paddies, vineyards and orchards can be locally abundant. Urbanized areas, including residential settlements as well as industrial and commercial zones, are widespread and distributed across much of the territory. This area is characterized by the presence of the Po River and its numerous tributaries, and it is strongly shaped by land reclamation and an extensive network of irrigation canals. Water bodies of various types (restored

natural areas, quarries, artificial ponds, and hunting reserves) are present and locally abundant; extensive wetlands are also present, especially toward the coast. The climate of this area is generally temperate continental, characterized by hot and dry summers and cold winters. Moving toward the Adriatic coast, the climate shows Mediterranean influences.

Mosquito sampling and morphological identification

Mosquitoes were sampled during the entomological surveillance of West Nile virus and Usutu virus, in the frame of the Regional plan, which implements action provided by the national Plan.

Mosquitoes were collected in the lowland area of the Emilia-Romagna region, in a network of traps evenly distributed in the surveyed area according to a 11 km^2 grid, with an average of 93 traps per season (minimum 64, maximum 109); in 2025, the average distance between more nearest traps was 8.1 km. Sites of sampling and employed traps (CDC-like traps baited by carbon dioxide) were chosen to maximise the collection of *Culex* mosquitoes, the main target of this surveillance. Every site was sampled from May to October, every two weeks; the trap was deployed in the evening and retrieved the following morning. Collected mosquitoes were transported in laboratory, killed by freezing and identified at species level according to morphological keys[11,12,35]. *Cx. perexiguus* was identified also considered morphological characters reported in other identification keys[6,7,10].

The annual total number of *Culex* mosquitoes and the number of traps in which each *Culex* species was collected were reported for each season. Seasonal mosquito abundance among positive trapping events was summarized using the geometric mean. This measure was chosen to reduce the influence of large catches, which typically affect the arithmetic mean. The occurrence of each mosquito species was considered separately and expressed as the number of sites where the species was collected in a given season. The seasonal dynamics of *Cx. pipiens* and *Cx. modestus* were summarized by fitting smoothed curves to mosquito captures (mosquitoes per trap per night) recorded through surveillance activities between 2001 and 2025, using the geom_smooth() function with the LOESS method. Figures were generated using the ggplot2 package in R[60].

Barcoding and phylogenetic analysis

Sequences of the COI were obtained starting from DNA extracted from two legs of the mosquito. Briefly, after removing two legs from each mosquito, these were homogenized in PBS (1X phosphate-buffered saline). Genomic DNA was extracted using the KingFisher Apex instruments magnetic particle processor (Thermo Fisher Scientific, Waltham, MA, USA). The DNA thus obtained was amplified following the protocol reported in Folmer et al. (1994)[61]. Obtained amplicons were sequenced using the Spectrum CE System (Promega) according to the manufacturer's instructions and deposited in BOLD database[62] with the ProcessIDs from MOER001-26 to MOER021-26.

The overall and pairwise p-distance (pairwise deletion) were obtained by MEGA 12[63]. Sequences available on BOLD database[62](accessed February 2, 2026) with a Barcode Index Numbers (BIN) referable to the Univittatus subgroup (at least one sequence in the BIN deposed as *Cx. perexiguus*, *Cx. univittatus*, *Cx. neavei*, *Cx. fuscocephala*), were retrieved. Only the BIN group in which the majority of sequences were attributed to a species of the group and with more than 3 sequences were then utilized; a sequence of *Cx. pipiens* obtained in this work and deposited in BOLD database (ProcessID MOER022-26) was used as outgroup. Selected sequences were aligned using MAFFT[64], identical sequences were excluded and the alignment were refined with MaxAlign program obtaining an alignment with 266 sequences. The maximum likelihood tree was obtained by IQTREE[65], which found the TIM2+F+R3 as the best-fit model; the sequence of *Cx. pipiens* were used as outgroup and the ultrafast bootstrap approximation as bootstrap option. The tree was visualized with iTOL[66].

Climatic data and modelling

The presence points of *Cx. perexiguus* were used to obtain the ENM in the study area according to the maximum entropy approach, using Maxent software v3.4.1[67]. This software estimates environmental suitability for a species departing from a set of occurrence locations and gridded covariates, maximising the entropy in geographic space or, in other terms, minimizing relative entropy between covariates. A series of bioclimatic and ecological variables (such as soil type and land use) were obtained from different sources (Table S3). All covariates were acquired as raster, then rescaled and aligned at 1 km^2 spatial resolution in ASCII format on the surveyed area extent (WGS84 projection) by using R package terra. Meteorological conditions were described by using the 2025 monthly averages of daily mean, minimum, maximum temperature and the 2025 monthly sums of precipitation, and the corresponding anomalies with respect to the period 2001–2020. The data were extracted from the ERG5 regional meteorological dataset, with a resolution of nearly 5 km^2 (https://dati.arpae.it/dataset/erg5-interpolazione-su-griglia-di-dati-meteo). Departing from meteorological data, bioclimatic indexes for the year 2025 were calculated by R package dismo.

The 75 available covariates were screened for correlation, selecting those having a Pearson's correlation coefficient of less than 0.7 in order to avoid potential collinearity problems, obtaining a subset of 54 covariates (Table S3). Correlation and collinearity analysis were performed in R and ENM Tools. The R package ENMeval was used to evaluate the best parameter settings in Maxent. Background was created using 10,000 random points, automatically generated by Maxent and we used a regularization 2. The output grid format was set to Cloglog. Minimum training threshold was adopted to convert maps from suitability indices to presence/absence (GPS data). To assess model accuracy, we used 10-fold cross-validation and we calculated the mean AUCs (Area Under Curve).

All the maps were created using the software QGIS 3.34[68].

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-026-76953-4.

Author contributions G.D., A.G., M.S., E.C. and F.S. performed the sampling and identified the mosquitoes; A.B.C. carried out the sequencing; A.A., G.A., and M.C. analyzed the data; M.C. wrote the manuscrip, all authors reviewed the manuscript.

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Data availability Sequence data generated in this study are available in the BOLD database under ProcessIDs (direct link): MOER001 (https://portal.boldsystems.org/record/MOER001-26), MOER002 (https://portal.boldsystems.org/record/MOER002-26), MOER003 (https://portal.boldsystems.org/record/MOER003-26), MOER004 (https://portal.boldsystems.org/record/MOER004-26), MOER005 (https://portal.boldsystems.org/record/MOER005-26), MOER006 (https://portal.boldsystems.org/record/MOER006-26), MOER007 (https://portal.boldsystems.org/record/MOER007-26), MOER008 (https://portal.boldsystems.org/record/MOER008-26), MOER009 (https://portal.boldsystems.org/record/MOER009-26), MOER010 (https://portal.boldsystems.org/record/MOER010-26), MOER011 (https://portal.boldsystems.org/record/MOER011-26), MOER012 (https://portal.boldsystems.org/record/MOER012-26), MOER013 (https://portal.boldsystems.org/record/MOER013-26), MOER014 (https://portal.boldsystems.org/record/MOER014-26), MOER015 (https://portal.boldsystems.org/record/MOER015-26), MOER016 (https://portal.boldsystems.org/record/MOER016-26), MOER017 (https://portal.boldsystems.org/record/MOER017-26), MOER018 (https://portal.boldsystems.org/record/MOER018-26), MOER019 (https://portal.boldsystems.org/record/MOER019-26), MOER020 (https://portal.boldsystems.org/record/MOER020-26), MOER021 (https://portal.boldsystems.org/record/MOER021-26), MOER022 (https://portal.boldsystems.org/record/MOER022-26),. Mosquito sampling data used to produce graphs and ENM modelling are provided in the Supplementary Materials.

Declarations

Competing interests The authors declare no competing interests.

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Authors and Affiliations

Mattia Calzolari[1] · Annalisa Grisendi[1] · Mara Scremin[1] · Gastone Dalmonte[1] · Emanuele Callegari[1] · Anna Beatrice Casano[1] · Francesco Severini[2] · Gabriele Antolini[3] · Alessandro Albieri[4] · Dottori Michele[1]

✉ Mattia Calzolari
mattia.calzolari@izsler.it

[1] Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna "B. Ubertini", Via Bianchi 9, Brescia 25124, Italy

[2] Istituto Superiore di Sanità, Viale Regina Elena 299, Roma 00161, Italy

[3] Arpae Emilia-Romagna, Via Po 5, Bologna 40139, Italy

[4] Centro Agricoltura Ambiente "G. Nicoli", Via Sant'Agata 835, Crevalcore (BO) 40014, Italy