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Evidence of expanding range of Culex perexiguus in Northern Italy (Emilia-Romagna, 2025)

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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 the distribution of 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 still 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 neave*i 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].

Cx. 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].

Cx. 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 [6,11,1213,14]. 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], 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)*

From about 100,000 to more than 400,000 *Culex* mosquitoes per season were collected from 2009 in the frame of the entomological WNV surveillance, in a number of traps ranging from 64 to 109 (Fig. 1, tab. S1). The more abundantly collected *Culex* species was *Cx. pipiens* followed by *Cx. modestus*, even with substantially lower numbers (Fig. 1).

The seasonal dynamic of *Culex 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. 1). The seasonal abundance of *Cx. pipiens* over the years, exemplified by geometric mean, seems to show a slightly decreasing trend, but this

mosquito was collected in all the monitored sites in all seasons. The mosquito *Cx. modestus* had become less abundant over time, with a reduction also in terms of sites in which the mosquito was sampled (Fig. 1).

On September 2025, a female mosquito bearing the *Cx. perexiguus* morphological characters was collected in a site in Imola municipality, Bologna province (Fig. 2, tab. S2). The smallest size, the postspiracular area with a pale scale patch[6,7], and a pale longitudinal stripe on the ventral part of the hind tibia[11] are the more evident characteristics distinguishing this species from *Cx. pipiens*, the morphologically more similar mosquito collected in the surveyed area. Moreover, *Cx. perexiguus* has a white line on the base of the costal vein[12] and the basal bands on abdominal terga, usually thinner than in *Cx. pipiens*, while the ring of pale scales on femora and tibias, sometimes reported as a distinctive character of *Cx. perexiguus*, could be present in some melanic specimens of *Cx. pipiens*. The general *habitus* of the two species are similar: the proboscis is dark with light scales, while the rest of the body is covered with golden and brown scales. The morphological differentiation between *Cx. perexiguus* and *Cx. univittatus* is a more difficult task, which can be done by looking at the length of the hind tibia stripe and the shape 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.

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 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 together with *Cx. perexiguus* sequences originating mainly from Africa, but also from Europe, particularly Spain, Italy, and Greece (Fig. 3). 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).

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 (tab. 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%) with 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%) (tab. 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 (fig. S1). Other 23 covariates also contributed to the ENM, but each accounted for less than 5% (tab. 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 (tab. 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 (Fig. 6). This pattern is likely attributable to the homogeneity of the surveyed area and does not seem to have influenced the occurrence of *Cx. perexiguus*. 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. 6). Additional thermal anomalies were observed in January, February, and March, especially in minimum temperatures, with increases ranging between + 2°C and + 3°C; conditions that may have been favourable for this species (Fig. 6).

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].

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 seasonal dynamics. 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. Other introduction pathways not linked to human-mediated transport therefore seem more plausible. 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]. On the contrary, in the northern part of its range *Cx. modestus* seems to have increased its abundance, with a signalled widened presence in Bohemia between 2004 and 2007[57], as well as the first records from England in 2010[58] and Belgium in 2018[59].

Taken together, these findings suggest a northward shift of the suitable area for both species, resulting in a gradual reduction of *Cx. modestus* in the study area and the potential establishment of *Cx. perexiguus*. This shift is possibly driven by climate warming, as new habitats become suitable for colonization in warming scenarios. As reported above, these observations seem to fit into a broader phenomenon of poleward shifting of several mosquito species, indicating a dynamic changing of its areal driven by climate change. If this phenomenon is confirmed, the change of mosquito fauna in a given area will produce new health risk scenarios that will need to be carefully monitored and characterized for adoption of appropriate public health actions.

The presence of *Cx. perexiguus* in the Emilia-Romagna region is a surprising observation, and the coming seasons will be crucial to determine whether this is 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 (minimum 64, maximum 109). 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, collected mosquitoes were transported in laboratory, killed by freezing and identified at species level according to morphological keys[11,12,35]. Morphological characters reported in other keys[6,7,10] were considered to identify *Cx. perexiguus*.

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)[60]. 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[61]. 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[63], 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[64], 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[65].

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[66]. 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 (tab. 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 km2 (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 (tab. 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[67].

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

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Author Contribution

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.

Data Availability

Sequence data generated in this study are available in the BOLD database under Sequence IDs MOER001-26.COI-5P; MOER002-26.COI-5P; MOER003-26.COI-5P; MOER004-26.COI-5P; MOER005-26.COI-5P; MOER006-26.COI-5P; MOER007-26.COI-5P; MOER008-26.COI-5P; MOER009-26.COI-5P; MOER010-26.COI-5P; MOER011-26.COI-5P; MOER012-26.COI-5P; MOER013-26.COI-5P; MOER014-26.COI-5P; MOER015-26.COI-5P; MOER016-26.COI-5P; MOER017-26.COI-5P; MOER018-26.COI-5P; MOER019-26.COI-5P; MOER020-26.COI-5P; MOER021-26.COI-5P; MOER022-26.COI-5P. Mosquito sampling data used to produce graphs and ENM modelling are provided in the Supplementary Materials.

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Figures

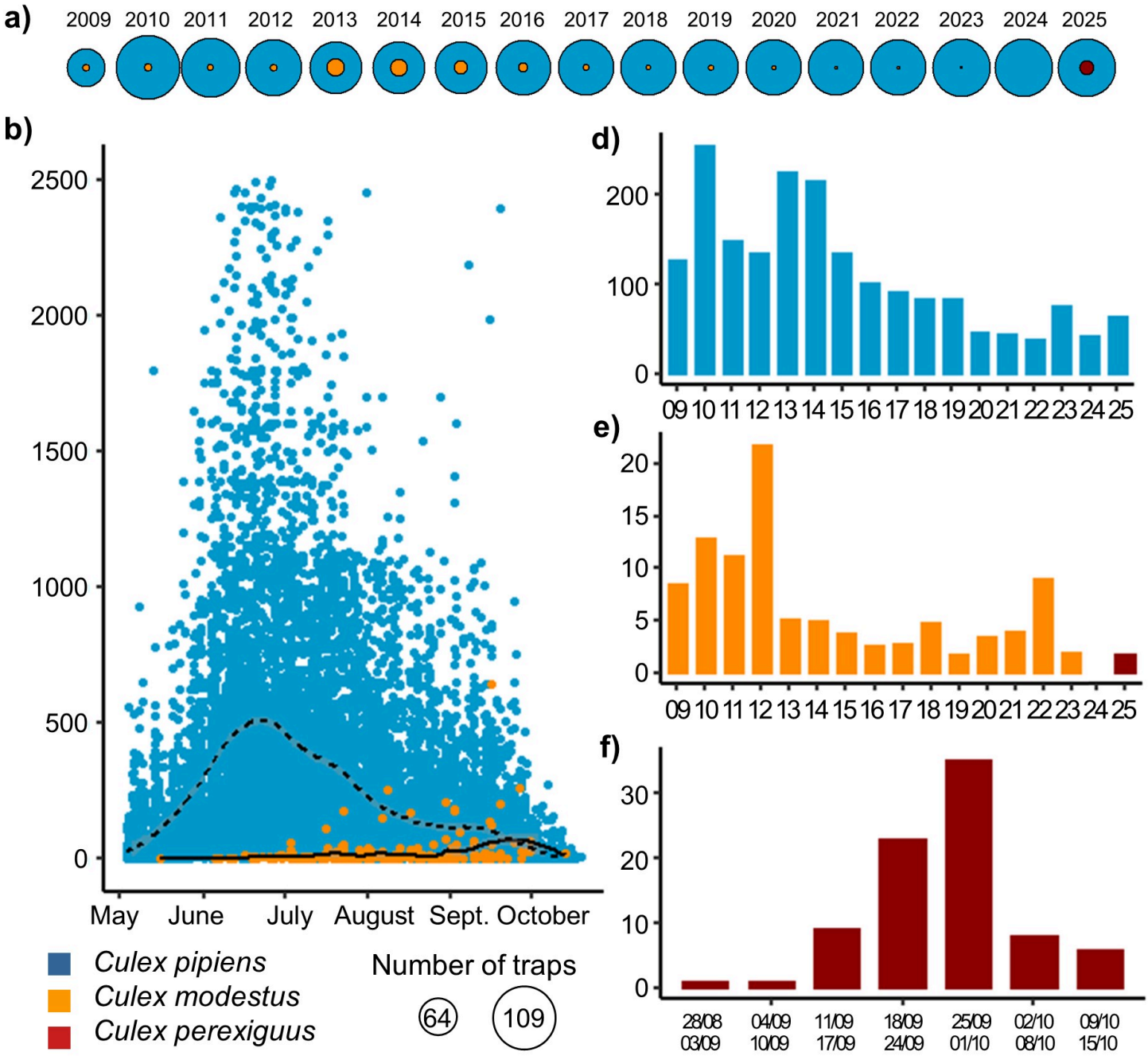


Figure 1

Entomological data referring to *Culex* mosquitoes, collected in the frame of Emilia-Romagna WNV surveillance, 2009-2025. Number of entomological traps working in the different seasons with reference to those in which the different mosquitoes were captured (Cx. pipiens were sampled in all traps every year) (a). Seasonal dynamics of *Cx. pipiens* and *Cx. modestus*exemplified by the interpolation line of all samplings performed during the period (b). Geometric mean of collected mosquitoes during the different year, *Cx. pipiens* (d) *Cx. modestus* and *Cx. perexiguus* (e). Total number of *Cx. perexiguus* mosquitoes collected on a weekly basis in 2025 (f).



Figure 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).

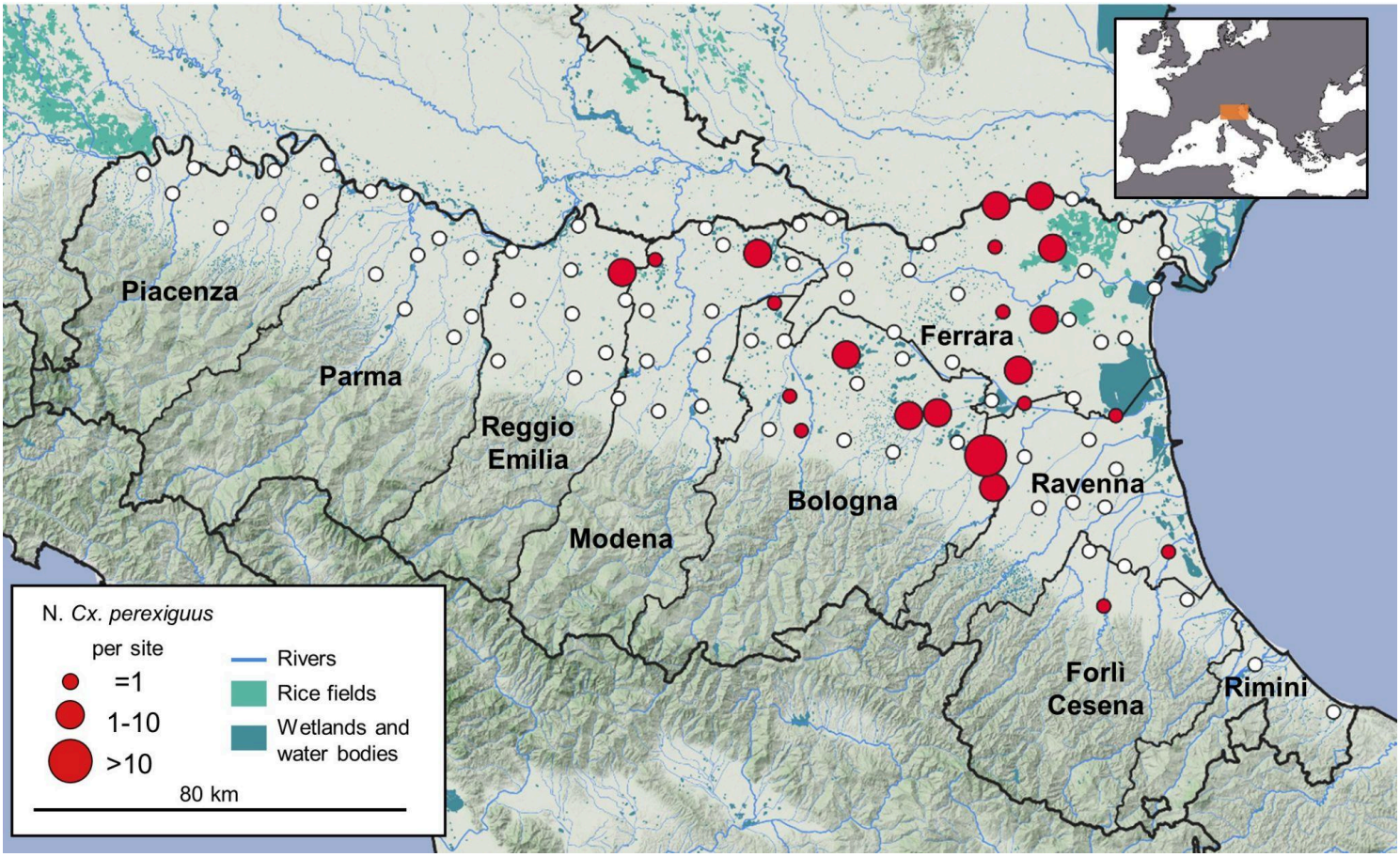


Figure 3

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 number of collected specimens.

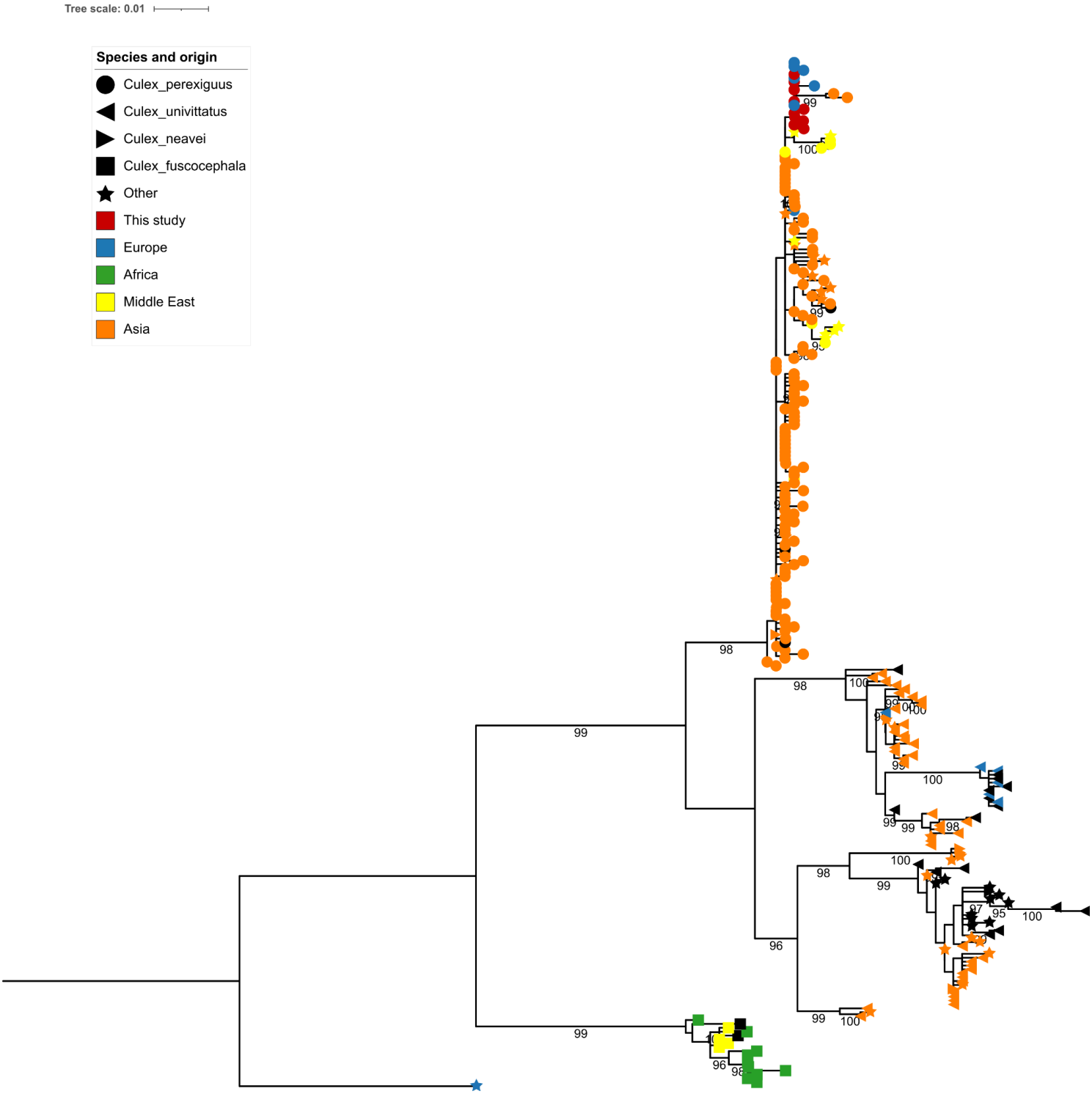


Figure 4

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; sequences from this study in red. Only bootstrap over 95% are shown near the branches.

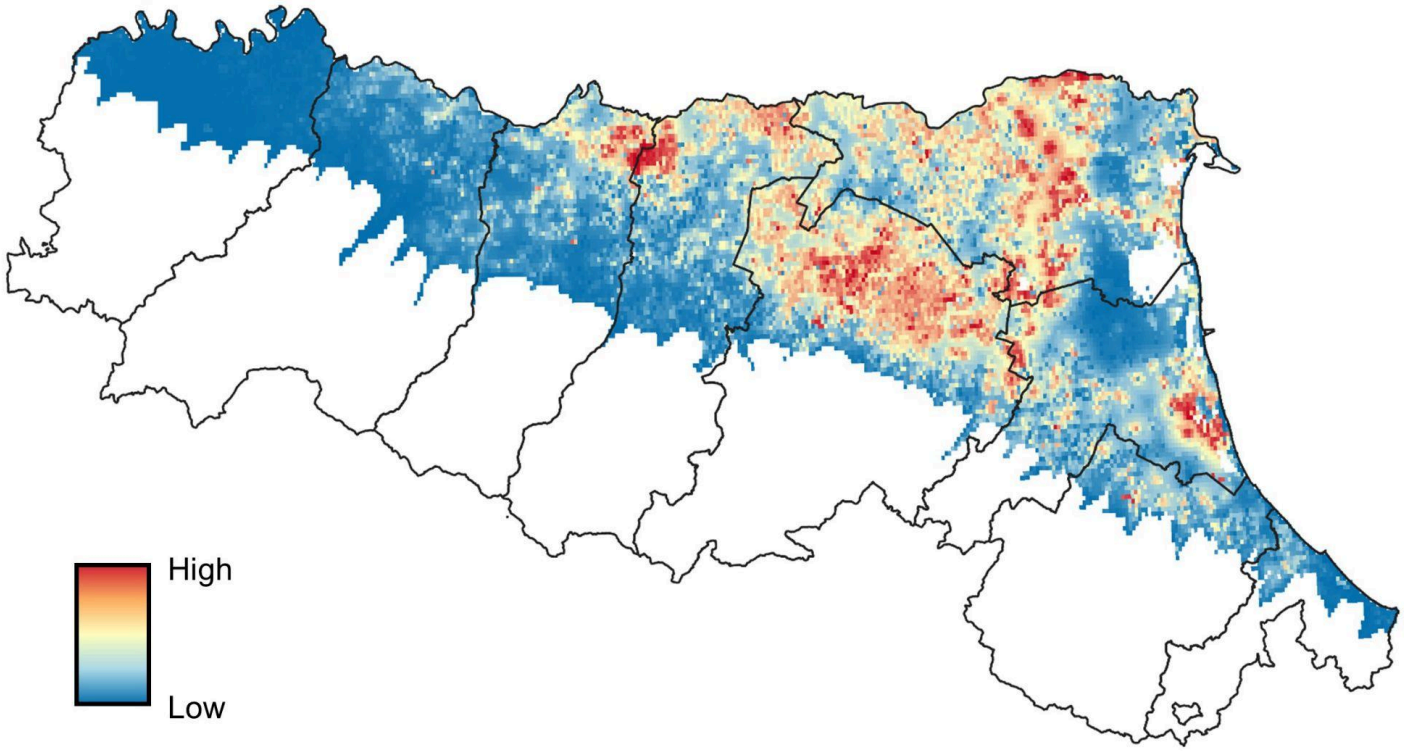


Figure 5

Predicted probability of suitable conditions for Cx. perexiguus obtained by Maxent model in the lowland of Emilia-Romagna.

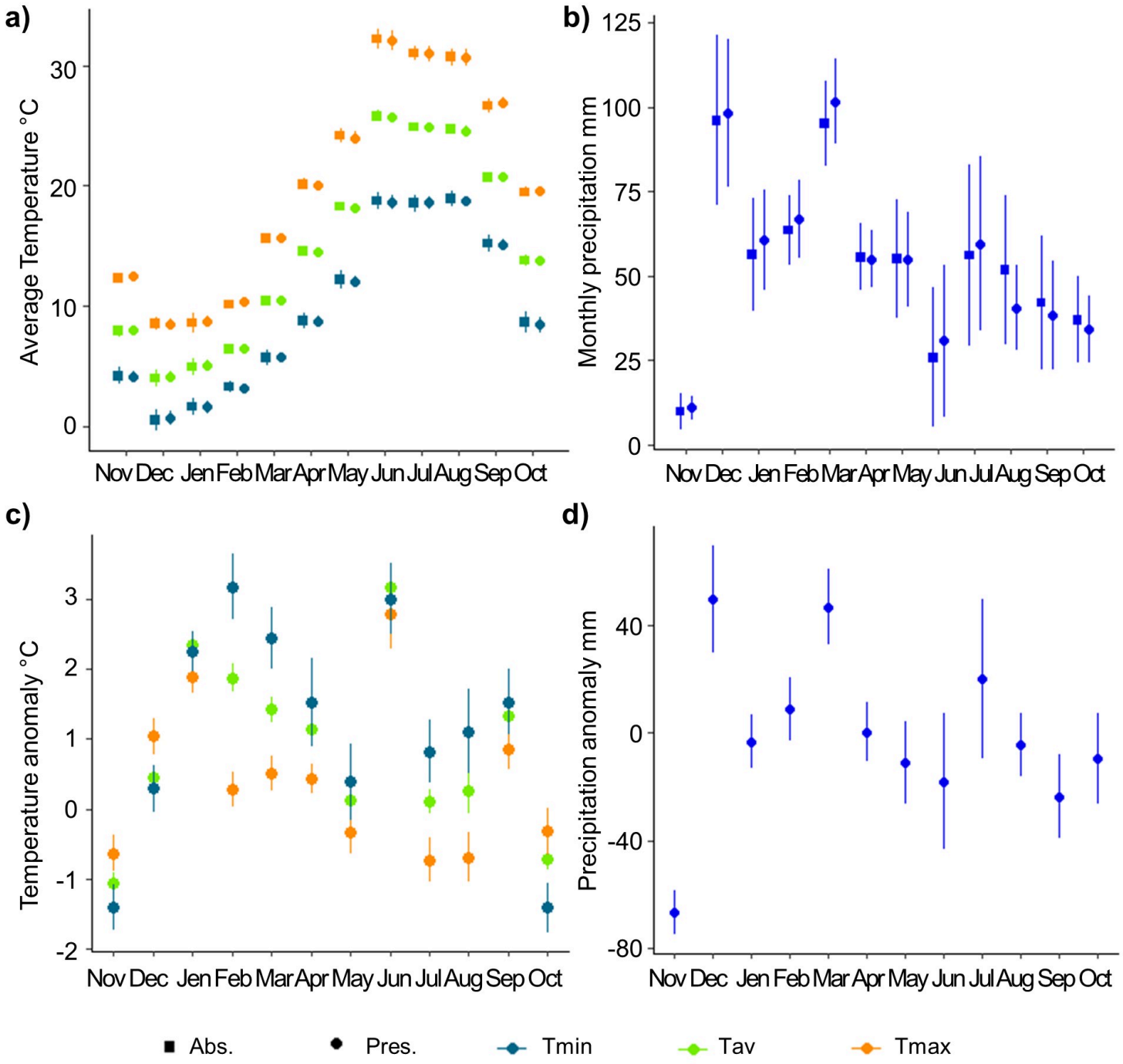


Figure 6

Meteorological conditions recorded from November 2024 to October 2025 in surveyed sites. Average of temperature (a) and precipitation (b) recorded in sites with presence and absence of *Cx. perexiguus*. Average of temperature (c) and precipitation (d) anomalies recorded in sites with presence of *Cx. perexiguus*.

Supplementary Files

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