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Knock-knock, who is inside? Exploiting citizen science data to shed light on *Aedes albopictus* indoor presence in southern Europe

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Abstract

1. Animal behaviour in human-dominated landscapes is difficult to study at large spatial and temporal scales, especially for small species characterised by elusive movements. Citizen science can help overcome these limitations by turning routine human–animal encounters into observations of where and when species are active. Here we exploited the Mosquito Alert citizen science platform to study the indoor versus outdoor presence of *Aedes albopictus* (generally considered as predominantly exophagic/exophilic) in contrast with sympatric *Culex pipiens*.
2. We analysed 21,433 expert-validated photographic records of *Ae. albopictus* and *Culex* spp. from Italy and Spain (2021–2024) classified by Mosquito Alert contributors as taken indoors or outdoors. We combined descriptive statistics with generalised linear and additive mixed models to (i) quantify species-specific probabilities of indoor records and (ii) estimate the relative contribution of *Ae. albopictus* to indoor mosquito records. We complemented the citizen science results with two small-scale indoors versus outdoors entomological collections carried out in Italy in August–September 2024.
3. Overall, 56% and 91% of *Ae. albopictus* and *Culex* spp. records were classified as indoor, respectively. The modelled probability that an *Ae. albopictus* record referred to a specimen photographed indoors averaged 56.3% (95% CI: 49–63) and increased

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from 40% to 50% in late spring to ~60% in late autumn. In contrast, the probability of *Culex* spp. indoor records remained consistently >90%. Among indoor records, the probability that a photographed specimen was *Ae. albopictus* averaged 61.8% (95% CI: 58.7–64.9) and peaked at 73.4% (95% CI: 70.6–76.0) in midsummer. Results from the small-scale field assessments show observed daily mean female *Ae. albopictus* indoor ratios of ~22% and ~27% in the two study sites.

4. These results provide a first estimate of indoor presence of *Ae. albopictus* in its invasive range in Europe. Even accounting for the biases associated with this citizen science data, results show that the probability of coming into contact with *Ae. albopictus* indoors is not negligible, with relevant implications for epidemiological studies, vector control and public health. Moreover, the study highlights the potential of citizen science platforms to act as observatories for wildlife living in close association with humans, revealing behavioural patterns not assessable otherwise.

KEYWORDS
Aedes albopictus, *Culex pipiens*, endophagy, endophily, Italy, mosquito, mosquito alert, Spain

1 | INTRODUCTION

Aedes albopictus (commonly known as the Asian tiger mosquito; Skuse, 1894) is the most invasive mosquito species worldwide. Its spread from its original range in South-East Asia to US, Europe and other continents has been facilitated by human-mediated transport, particularly through the international trade of goods such as used tires and ornamental plants that provide suitable larval habitats (Eritja et al., 2017; Lucati et al., 2022; Swan et al., 2022). In Europe, the species was first detected in Albania in the late 1970s (Adhami & Reiter, 1998) and in Italy in 1990 (Sabatini et al., 1990). Nowadays, the species is established in all Mediterranean countries and still expanding northwards and eastwards (https://www.ecdc.europa.eu/en/publications-data/aedes-albopictus-current-known-distribution-june-2025). The increasing number of autochthonous transmission and outbreaks of chikungunya and dengue occurred in the last decade in Italy, France and Spain (https://www.ecdc.europa.eu/en/all-topics-z/dengue/surveillance-and-disease-data/autochthonous-transmission-dengue-virus-eueea-previous-years; https://www.ecdc.europa.eu/en/infectious-disease-topics/chikungunya-virus-disease/surveillance-and-updates/local-transmission-previous-years) highlights the risk of a significant public health burden in future years in the whole Mediterranean region.

In its native tropical range in Asia, *Ae. albopictus* exhibits predominantly exophagic and exophilic behaviour, typically feeding and resting outdoors (Guo et al., 2025; Hawley, 1988). Few studies report occasional indoor resting or biting incidents in Malaysia, Indonesia, Sri Lanka (Dieng et al., 2010; Haziqah-Rashid et al., 2019; Herath et al., 2024), suggesting that under certain ecological or climatic conditions, *Ae. albopictus* may exploit indoor environments. Because of its strong preferences for outdoor environments in the native range and its generalist feeding behaviour (Cebrián-Camisón et al., 2020; Valerio et al., 2010), the species is generally considered exophagic and exophilic also in temperate regions. However, compared to the native range, the invasive European range presents cooler temperatures and higher seasonal variability, which could influence mosquito behaviour and potentially increase interaction with indoor environments. Conventional surveillance in Europe relies on outdoor collections by ovitraps and adult traps deployed in public urban/peri-urban areas (European Centre for Disease Prevention and Control, 2012; Schaffner et al., 2013) and is not appropriate to highlight a possible behavioural shift from the outdoor to the indoor environment. Thus, so far only few evidence suggests a possible weaker exophagy/exophily of *Ae. albopictus* in temperate regions. Sampling carried out in Rome (Italy) showed that while most females were collected outdoors, blood-fed females were mostly collected indoors (Valerio et al., 2010). Consistently, a recent large-scale study targeting resting adults in France failed to collect blood-fed females, which could be at least partially due to indoor feeding and resting in the first digestion phase (Clauzon et al., 2026). Understanding and quantifying *Ae. albopictus*' actual indoor activity has practical relevance, as indoor biting could affect arboviral transmission risk, potentially undermining the effectiveness of control strategies that target outdoor breeding and resting sites.

Citizen science—the active involvement of non-professional participants in scientific research and knowledge production (Socientize Consortium, 2013)—has become an increasingly valuable tool in environmental and ecological sciences, increasing the knowledge about biodiversity all over the world (Fraisl et al., 2022). By leveraging modern technologies such as smartphones, GPS and real-time data sharing, citizen science allows the collection of ecological

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information across broad spatial and temporal scales that would be difficult or prohibitively expensive to achieve through traditional surveillance approaches alone. In Europe, several mosquito-focused citizen science projects have been developed over the past decade (Braz Sousa, Craig, et al., 2022a), using mobile applications to collect photographic records, bite reports or even physical specimens from private and public areas. These initiatives have generated extensive datasets on mosquito presence, abundance and seasonal dynamics, complementing professional surveillance networks and filling critical gaps in areas that are logistically difficult to access (Braz Sousa, Fricker, et al., 2022b; Caputo et al., 2020; Walther & Kampen, 2017). These datasets also contain underexplored information that could be used to investigate mosquito behavioural aspects—such as indoor versus outdoor activity, biting patterns and changes in habitat preferences or host-seeking behaviour (Abourashed et al., 2025; Cull et al., 2024). Among these projects, Mosquito Alert (MA; www.mosquitoalert.com; www.mosquitoalertitalia.it) has emerged as the leading citizen science platform on medically important mosquito species in Europe (Južnič-Zonta et al., 2022). Mosquito Alert collects photographic records of *Aedes* invasive mosquitoes and of the native *Culex pipiens*, as well as photos of breeding sites and reports of biting activity, from across the continent and beyond. Each submission of a mosquito photographic record is validated by trained entomologists, ensuring a high level of data quality.

In this study, we use expert-validated photographic records collected through the Mosquito Alert app between 2021 and 2024 to investigate the spatio-temporal dynamics of *Ae. albopictus* in Italy and Spain, with a specific focus on its occurrence indoors relative to outdoors. We compare these patterns to those of *Culex pipiens*, a native European species with strong indoor biting activity, to assess species-specific differences in indoor presence and to explore their implications for nuisance levels and arboviral transmission risk.

2 | MATERIALS AND METHODS

2.1 | Mosquito alert ITALIA and SPAIN data and samples

Mosquito Alert is a citizen science initiative that engages volunteers in the surveillance of mosquito species of public health relevance, including the invasive *Ae. albopictus* and the native *Culex* spp. Using a free and open-source smartphone application available for Android and iOS (www.mosquitoalert.com; www.mosquitoalertitalia.it; https://github.com/Mosquito-Alert/Mosquito-Alert-Mobile-App) participants submit georeferenced reports of adult mosquitoes, mosquito bites and mosquito breeding sites. Reports of adult mosquitoes are later validated by expert entomologists in the Mosquito Alert Digital Entolab (see File S1). References cited only in the Supporting Information are listed in the Data sources section.

For the present study, a database (hereafter DB) of expert-validated georeferenced mosquito photos from the publicly available Mosquito Alert database (hereafter, MA records) was compiled with records transmitted from Italy and Spain by citizen scientists during the main period of adult activity of both species, which lasts from the second half of April to the first half of November (Becker et al., 2010; Medlock et al., 2012) for the years 2021 through 2024 (File S1; Figure S1; note that there is a temporal gap in this data from 16 to 26 July 2022, due to a technical issue that blocked reporting). Only photos identified as *Ae. albopictus* and *Culex* spp. by Mosquito Alert Entolab experts were included in the DB. For the specific purposes of this study, *Culex* spp. specimens are considered as *Cx. pipiens*, as this is the predominant species of the genus in Southern Europe (Becker et al., 2012). Each MA-record was classified as taken 'in a building' (hereafter, indoors) or 'outdoors' based on citizen scientist input provided through a mandatory selection step during the Mosquito Alert submission process. Records reported as 'in a vehicle' were excluded as they were very few in number and fell outside the scope of this study. A random subset of records was visually inspected by expert entomologists to determine the sex of the depicted specimen on the basis of diagnostic morphological characters (antennal plumosity, maxillary palp length and terminalia) (Becker et al., 2010).

Mosquito Alert records were mapped onto a spatial grid covering the two countries with the same resolution as the MA sampling cells (0.025° longitude and latitude, which is approximately 4 km^2 on the ground in this region). The grid was generated in R by creating a regular lattice of square polygons from masked background locations of participants, cropped to the national boundaries and joined with polygons of the administrative units. Each cell is associated with (i) administrative information, (ii) cell centroid coordinates and (iii) a unique identifier. These attributes allowed spatial linking of each MA-record to the grid structure, by a unique grid cell identifier already present in the MA downloaded data. Records were not aggregated by grid but simply associated with information contained in the grid cells. R packages employed for data processing were sf (v 1.0.19; Pebesma, 2018) and sp (v 2.2.0; Pebesma & Bivand, 2005).

To account for differences in MA app usage by citizen scientists across space and time, the Sampling Effort (SE) was computed for each cell and each 2-week period (hereafter biweek) as the sum of participants who downloaded the app and consented to the activation of SE, weighted by each participant's reporting propensity score at the time of the sighting (Palmer et al., 2017; Padilla-Pozo et al., 2024; Virgillito et al., 2024; File S1). To minimise the disproportionate influence of a small subset of highly active participants, SE outliers in the DB were identified and excluded following Zuur et al. (2010) (File S2; Figure S2, sensitivity analysis in File S3—Table S1). Similar approaches have been adopted in large-scale citizen science initiatives—for example eBird routinely filters large checklists to normalise observer effort (Sullivan et al., 2014), and semistructured survey designs on platforms such as iNaturalist implement effort-based thresholds to reduce bias (Kelling et al., 2019). In our dataset, SE distribution was heavily right-skewed, with a long tail of high-effort outliers. We therefore applied the interquartile range (IQR) method (Tukey, 1977) to define and exclude upper outliers, retaining only records representative of typical user activity.

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2.2 | Mosquito alert data quality checks

Data quality checks were performed to evaluate the temporal and spatial structure of the data and its suitability for the statistical analyses (File S4).

2.2.1 | Reporting intensity versus sampling effort

For each grid cell, the number of observed MA records (hereafter MA-record intensity) was compared to the cumulative SE, defined as the sum of SE values across all records within that cell. Both variables were $\log_{10}$-transformed and visualised using hexagonal binning (50 bins; Figure S3). This step was necessary to account for observation bias, as in citizen science datasets the number of records is inherently driven by spatial coverage and SE (Palmer et al., 2017).

2.2.2 | Temporal metadata accuracy

The timestamp, defined as the digital record of the date and time when a photo was taken (stored in its EXIF metadata), was compared with the record creation time across multiple temporal scales (1-h, 2-h, 1-week and 2-weeks). The validation was performed through repeated random subsampling of the dataset (100 iterations of 500 randomly selected records per iteration) to estimate the consistency and robustness of the temporal agreement between timestamp and MA records creation time (File S4). Although conceptually similar to bootstrapping, this procedure did not involve sampling with replacement and was therefore used to evaluate stability rather than to derive confidence intervals from resampled distributions.

2.2.3 | Spatial autocorrelation

Spatial clustering of MA-record intensity was evaluated by calculating Moran's I on Pearson residuals from a Bayesian negative-binomial model of MA-record intensity versus SE (Bivand et al., 2013; Dormann et al., 2007), using a 4-nearest-neighbour spatial network (File S4). Although significant positive spatial autocorrelation was observed (Moran's $I = 0.069$, $p < 0.001$), spatial random fields were excluded to preserve model parsimony and emphasise temporal and species level effects (Dormann et al., 2007). Excluding spatial terms may slightly inflate type I errors or residual dependence if strong spatial clustering were present; however, this was considered acceptable given the relatively weak spatial autocorrelation detected and the absence of explicit spatial predictions.

Temporal autocorrelation was partially addressed through the inclusion of a cubic spline on time in models, which flexibly captured seasonal and long-term temporal patterns. This approach acted as a control for temporal variation and served as a quality check for potential serial dependence.

2.3 | Statistical analysis

In addition to compiling descriptive statistics of counts, temporal dynamics and spatial distribution of the *Ae. albopictus* and *Cx. pipiens* georeferenced photographic records, we used Chi-squared and *t*-tests to assess differences in the location, year and seasonality of observed data of *Ae. albopictus* and *Cx. pipiens*. We used a regression modelling approach to analyse the probability of indoor versus outdoor reporting between and within the two species as specified below. Prior to statistical analysis, we standardised biweek and SE (Schielzeth, 2010; Zuur et al., 2010) by subtracting their mean value and dividing by their standard deviation. Used databases are described in File S5—Table S2 and in File S6—Table S3.

2.3.1 | Species-specific indoor versus outdoor presence probability (MODEL-1)

To quantify the species-specific probability of reporting an indoor versus outdoor mosquito, individual records obtained from April to November were retained and mapped to their corresponding species–cell–period–location. We then fitted a generalised linear mixed model (GLMM) with a Bernoulli logit link (hereafter MODEL-1), using the glmer function in the lme4 package (v. 1.1.36; Bates et al., 2015). Specifically, we modelled the log-odds of an indoor contact by defining the response variable for each individual record as a binary outcome (location coded as: indoor = 1, outdoor = 0). The full model included as fixed effects: species (*Ae. albopictus* vs. *Cx. pipiens*), a natural cubic spline of standardised biweek (df = 4) to capture non-linear intra-seasonal variation, standardised SE and the interaction between species and biweek; year and country were included as random intercepts to account for inter-annual and international heterogeneity in reporting. A stepwise model selection was performed on the full model using the dredge function from the MuMIn package (v. 1.48.4; Bartoń, 2025). Submodels were ranked based on corrected Akaike information criterion (AICc) values to select the most parsimonious model (Table S4).

The best-performing model (MODEL-1best) included the fixed effects of species and a natural cubic spline of standardised biweek (df = 4) to capture temporal variation, along with random intercepts for year and country.

2.3.2 | Frequency of indoor presence of *Ae. albopictus* relative to *Cx. pipiens* (MODEL-2)

To quantify the probability that an indoor record was *Ae. albopictus* rather than *Cx. pipiens*, data were first aggregated to compute species-specific counts per cell-biweek-species. We then fitted a generalised additive mixed model (GAMM) with a binomial logit link (hereafter MODEL-2). The model was fitted using the gam function in the mgcv package (v. 1.9.1; Wood, 2011) in R. Specifically, for each aggregated sampling unit, the response variable was structured as a binomial outcome using the paired counts

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of *Ae. albopictus* (defined as successes) and *Cx. pipiens* (defined as failures). The full model included as fixed effects standardised SE and a smooth term for standardised biweek and as random-effect smooth year and country. A stepwise model selection was performed by iteratively comparing candidate models based on corrected Akaike Information Criterion (AICc) values (Table S5).

The best-performing model (MODEL-2best) included a smooth term for standardised biweek to capture temporal variation, and a random effect for year, modelled as a random smooth.

All analyses were conducted using the statistical software R, version 4.4.2 (R Core Team, 2024).

2.4 | Entomological assessment of mosquito indoor versus outdoor activity

A preliminary field experiment was conducted in two urban/suburban areas in Italy in 2024 from 26 August to 6 September in Rome (Lazio Region; 41°54′12″ N, 12°30′51″ E, hereafter Site-1) and from 30 August to 6 September in Padua (Veneto Region; 45°20′55″ N, 11°57′14″ E; hereafter Site-2). Collections were performed by two BG-Mosquitaire traps (BGM; Biogents AG, Regensburg, Germany) per site; each BGM was equipped with BG-Sweetscent chemical attractant (Biogents AG) and with an VECTRACK optical sensor (VECT; Irideon SL, Barcelona, Spain; www.irideon.es). The VECT sensor combines optical detection with a supervised machine learning algorithm for the automatic counting and genus- and sex-level identification of *Aedes* and *Culex* mosquitoes (González-Pérez et al., 2022). The accuracy of the species/sex identification was assessed in four sites in Spain and Italy showing high correlation between visual and automatic identifications, as well as a balance accuracy >90% for *Ae. albopictus* females (González-Pérez et al., 2024; Micocci et al., 2024). VECT also recorded temperatures at the time of mosquito capture (mean daily temperatures: 27°C–31°C in Rome; 22°C–30°C in Padua). At each site, one BGM + VECT trap was placed outdoors, under a canopy in proximity to vegetation, shelter from direct sun and rain, while the other was positioned indoors in an uninhabited building with open windows and doors. The two traps were positioned on the ground about 10 m apart and connected to an electrical power source. For the purpose of this study, only *Ae. albopictus* female specimens identified and counted based on the VECT system were considered.

2.5 | Ethics statement

This study used data collected through the Mosquito Alert citizen science platform. All participants provided informed consent within the app before submitting any data, in accordance with the Mosquito Alert terms of use and privacy policy and with the EU General Data Protection Regulation (Regulation (EU) 2016/679). Participation was voluntary and anonymous: no personal identifying information was collected, and each participant is linked to their reports only through a random anonymous identifier. Background location data used to compute sampling effort were collected only from participants who explicitly consented to this option. To protect participants' privacy, the app rounds these locations to a 0.025° grid (~4 km^2) before they leave the device. Access to these non-public data was granted by the Mosquito Alert coordinating team under a data-sharing agreement, and the data were analysed only in aggregated form.

The field collections involved only mosquitoes (Diptera: Culicidae), which are invertebrates. Mosquitoes are not covered by EU legislation on the protection of animals used for scientific purposes (Directive 2010/63/EU, implemented in Italy by Legislative Decree 26/2014), so no animal ethics approval was required. Traps were baited with a synthetic attractant (BG-Sweetscent), and no human or animal baits were used. The indoor traps were placed in uninhabited buildings with the permission of the owners/managers. The Culex specimens used for molecular identification were sent to Sapienza University voluntarily by Mosquito Alert participants.

3 | RESULTS

A total of 21,433 photographic records of *Ae. albopictus* and *Cx. pipiens* were received between 1 January 2021 and 31 December 2024. Unambiguous morphological examination of a subsample of 1654 photographic records showed that 91.4% of the examined specimens were females. We found that observed reporting intensity was positively associated with cumulative SE across grid cells (log_{10}-transformed values), as revealed by hexagonal binning (50 bins) (Pearson's $r = 0.81$, 95% CI: 0.80–0.82, $p < 0.001$; Figure S3). SE was therefore retained as the preferred proxy for observation bias in subsequent models, as it explicitly accounts for the number of active observers and their activity level rather than only the number of records; we also found high concordance between record creation timestamps and photo EXIF data, which captured 43.1% of the records with ±1-h tolerance (95% CI: 42.06–43.57) and 89.8% with ±2-h tolerance (95% CI: 89.46–90.12). At broader temporal resolutions, 96.6% of timestamps matched the calendar week (95% CI: 96.39–96.76), and 98.1% fell within the same biweekly period (95% CI: 97.94–98.34). In addition, we found significant but limited clustering of report intensity, as revealed by spatial autocorrelation analysis of grid-level residuals from a negative-binomial model (Moran's $I = 0.069$, $p < 0.001$).

The distribution of records among the four sampling years differed significantly between species ($\chi^2 = 17$, $p < 0.001$), although records of both species peaked in 2023 (36.9% and 37.3% of records, respectively). The monthly distribution of records differed significantly between *Ae. albopictus* and *Cx. pipiens* ($\chi^2 = 594$, $p < 0.001$): *Ae. albopictus* records were predominantly submitted during the summer months (June–September, 80.1%), whereas *Cx. pipiens* records were more evenly distributed across spring and summer, peaking in June (23.9%) and showing substantial activity in May (9.7%) and October (17.6%). Mean SE was similar for both species' records (*t*-test = 0.935, $p = 0.3498$).

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Overall, records of the two species were sent from a total of 5746 unique sampling cells with a coverage of 2.7% for *Ae. albopictus* and 1.3% for *Cx. pipiens* of the total grid (N_{tot} cells = 141,514) (Table 1), covering approximately 23,994 and 11,919 km^2, respectively, out of the total Italian-Spanish area of ~884,463 km^2. The spatial distributions of *Ae. albopictus* and *Cx. pipiens* records were broadly similar, with the majority of records of both species reported inland in both countries and about 20% of records reported in cells within 3 km from the coastline (File S7; Figure 1).

A higher proportion of expert-validated photographic records (55.8% and 91.4% of *Ae. albopictus* and *Cx. pipiens*, respectively) was taken indoors ($\chi^2 = 2267.2$, p-value = <0.001; Table 1). The temporal trend in indoor versus outdoor reporting from late April to early November (Figure S4) showed a progressive increase in the mean observed proportion of *Ae. albopictus* indoor records averaged across the four sampling years, rising from ~50% (95% CI 46.7–54.4) in June to 66% (95% CI 61.2–68.3) in October. In contrast, the mean observed proportion of *Cx. pipiens* indoor records was consistently >90%.

3.1 | How does the probability of *Ae. albopictus* records differ indoors versus outdoors?

MODEL-1 was aimed to assess the probability of receiving an indoor versus outdoor record of *Ae. albopictus* and *Cx. pipiens* from April to November. The most parsimonious model (MODEL-1best) included the fixed effect of species, a natural cubic spline for standardised biweek to capture temporal trends and random intercepts for year and country, which yielded the lowest AIC among all candidate models (Table S4). Standardised SE had no measurable effect on the probability of indoor mosquito reporting and was excluded. Overall, the predicted probability of *Ae. albopictus* records being indoors was 56.3% (95% CI: 49–63). A non-linear temporal pattern was estimated for *Ae. albopictus*, with probabilities to receive an indoor record ranging from 42% (95% CI: 31–54) in early May to 61% (95% CI: 50–71) in early November (Figure 2a). On the other hand, 90% (95% CI: 87–93) of *Cx. pipiens* photos were estimated to be taken indoors. Random intercepts for year and country accounted for minimal variance (SD = 0.21; SD = 0.053 respectively, $p > 0.20$) (Table S6).

3.2 | How does the probability of indoor reporting differ between *Ae. albopictus* and *Cx. pipiens*?

MODEL-2 was aimed to assess the probability of *Ae. albopictus* as opposed to *Cx. pipiens* indoor records from April to November as a function of location and SE. The model reveals distinct spatial–temporal patterns in the relative frequency of MA records of the two species. The most parsimonious model (MODEL-2best) included a smooth term for standardised biweek to capture

Characteristic	*Ae. albopictus*, N = 15,956	*Cx. pipiens*, N = 5477	*p*-Value
Year			<0.001
2021	2312 (14.5%)	900 (16.4%)	
2022	2775 (17.4%)	941 (17.2%)	
2023	5882 (36.9%)	2045 (37.3%)	
2024	4987 (31.2%)	1591 (29.1%)	
Month			<0.001
Late April	58 (0.3%)	72 (1.3%)	
May	585 (3.7%)	530 (9.7%)	
June	2723 (17.1%)	1310 (23.9%)	
July	3314 (20.8%)	1059 (19.3%)	
August	3427 (21.5%)	704 (12.9%)	
September	3311 (20.7%)	690 (12.6%)	
October	2420 (15.2%)	965 (17.6%)	
Early November	118 (0.7%)	147 (2.7%)	
Location			<0.001
Indoor	8903 (55.8%)	5006 (91.4%)	
Outdoor	7053 (44.2%)	471 (8.6%)	
Number of unique sampling cell (N = 141,514)[a]	3839 (2.7%)	1907 (1.3%)	
Mean sampling effort	0.192 (0.190, 0.193)	0.190 (0.187, 0.193)	

[a]Of these sampling cells N = 55,280 are in Italy (ITA) and N = 86,234 are in Spain (ES).

TABLE 1 Descriptive summary of validated *Ae. albopictus* (N = 15,956) and *Cx. pipiens* (N = 5477) Mosquito Alert photographic records from Italy and Spain (April–November 2021–2024). Rows report the distribution of records by year, month and observation location (indoor vs. outdoor). The number of unique 2.5 × 2.5 km sampling cells where records were reported is also shown, together with mean sampling effort per species record (mean, standard deviation). All *p*-values are derived from Pearson's chi-squared tests comparing species distributions across each characteristic.

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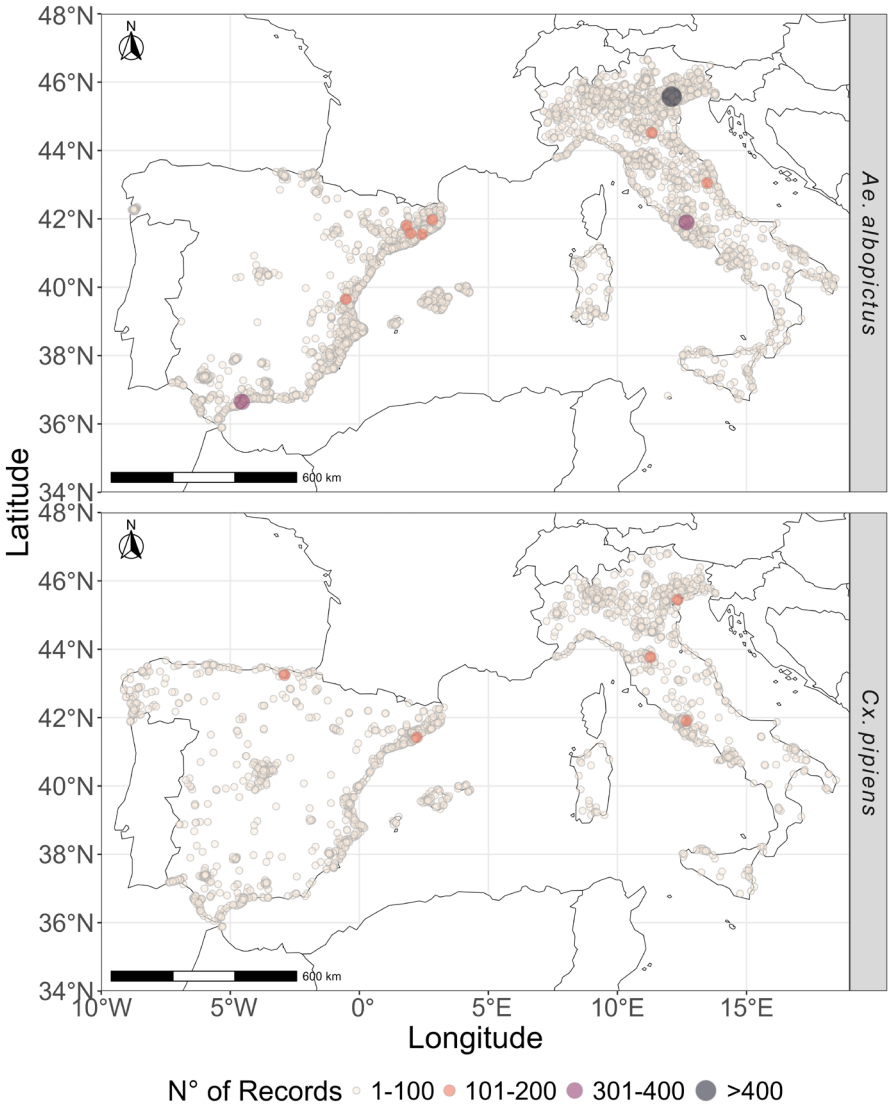


FIGURE 1 Distribution of Mosquito Alert photographic records of *Ae. albopictus* (upper panel) and *Cx. pipiens* (bottom panel) from Italy and Spain (April to November 2021–2024). Each dot represents the centroid of a 2.5 × 2.5 km grid cell, with dot size and colour scaled according to the total number of Mosquito Alert records aggregated within that cell.

temporal variation and a random effect for year modelled as a random smooth, which yielded the lowest AIC among all tested candidate models (Table S5). The estimated proportion of *Ae. albopictus* indoor records compared to *Cx. pipiens* averaged 61.8% (95% CI: 58.7–64.9) (Figure 2b). The temporal dynamics showed a pronounced curvilinear pattern, with a peak in the relative indoor activity of *Ae. albopictus* compared to *Cx. pipiens* in midsummer (July–August; 73.4%, 95% CI: 70.6–76.0) (Table S7). Standardised SE had no measurable effect on the relative frequency of *Ae. albopictus* relative to *Cx. pipiens* and was excluded from the final model during model selection. Random intercepts for year accounted for limited variance (SD = 0.110; $\chi^2 = 18.98$, $p = 8.78 \times 10^{-5}$), indicating small but significant inter-annual variation.

3.3 | Do field data support *Ae. albopictus* presence indoors?

A total of 690 *Ae. albopictus* females were collected and counted by the BGM-VEC system in the two small-scale field assessments of indoor host-seeking activity carried out in 2024 in Italy, to compare collections in host-seeking traps located in a building (148 and 54 in Rome and Padua, respectively) to those in traps located in neighbouring sheltered outdoor locations (393 and 95). The observed daily mean proportion of female *Ae. albopictus* captured indoors (calculated out of the total daily indoor and outdoor catches) was 27% (95% CI: 14.5–48.0; maximum indoor proportion 56%) and 22% (95% CI: 14.4–54.9; maximum indoor proportion 67%) in Rome and Padua, respectively (Figure S5).

4 | DISCUSSION

Aedes albopictus is assumed to be largely exophagic/esophilic based on data from the native tropical range, where it is often compared to the strongly endophagic/endophilic behaviour of the main arbovirus vector species, *Ae. aegypti* (Becker et al., 2010; Delatte et al., 2010; Egid et al., 2022; Petrić et al., 2014). However, very limited data are available on actual indoor/outdoor activity/presence of *Ae. albopictus* in its invasive range in temperate regions, where entomological

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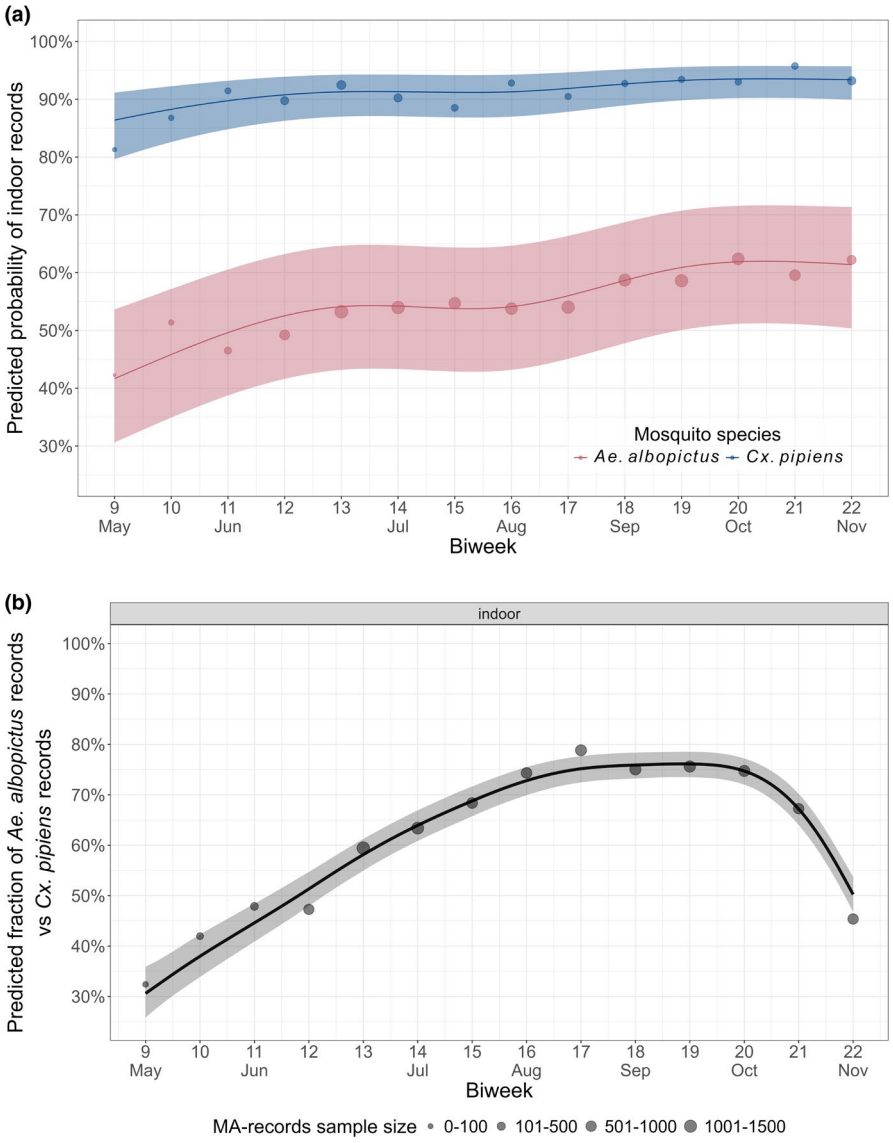


FIGURE 2 (a) Predictions from a generalised linear mixed model (GLMM; MODEL-1best) estimating the probability that a Mosquito Alert photographic record is reported indoors (vs. outdoors) for *Ae. albopictus* and *Cx. pipiens* from April to November. Records were collected via the Mosquito Alert smartphone application in Italy and Spain from 2021 to 2024. Observed data points represent the aggregation of all records per 2-week period (biweek) across years, with point size proportional to the total number of records. Model predictions are shown as lines, with shaded areas representing 95% confidence intervals. (b) Predictions from a generalised additive mixed model (GAMM; MODEL-2best) estimating the proportion of indoor Mosquito Alert photographic records attributed to *Ae. albopictus* relative to *Cx. pipiens* from April to November. Records were collected via the Mosquito Alert smartphone application in Italy and Spain from 2021 to 2024. Observed data points represent the aggregation of all records per 2-week period (biweek) across years, with point size proportional to the total number of records. Model predictions are shown as lines, with shaded areas representing 95% confidence intervals.

monitoring of the species for research or public health reasons is exclusively focused on the outdoor environment (European Centre for Disease Prevention and Control, 2012). The present study provides a first estimate of indoor presence of *Ae. albopictus* in southern Europe based on multiple-year citizen science data, that is on 21,433 expert-validated *Ae. albopictus* or *Cx. pipiens* photographic records sent by Mosquito Alert users from Italy and Spain.

Overall, 55.8% of the 15,956 *Ae. albopictus* and 91.4% of the 5477 *Cx. pipiens* photographic records were reported to have been taken in indoor environments, respectively. Results from MODEL-1, carried out to assess intra-species variations indoors versus outdoors, show that the estimated probability to find *Ae. albopictus* indoors increases from 42% in May to 61% in October, while it is always >90% for *Cx. pipiens*. While these features are consistent with the expected stronger endophagic/endophilic behaviour of *Cx. pipiens* (see below comments on *Cx. p. molestus*), they are in contrast with the general conviction on the *Ae. albopictus* strong exophagic/exophilic behaviour. Moreover, model results suggest that *Ae. albopictus* may increase its presence indoors in early autumn compared to summer. However, these estimates may be biased due to the greater difficulty of collecting or photographing mosquitoes outdoors compared with indoors, as well as by differences in how much time citizens spend inside versus outside, factors that coarse SE estimates do not capture and that we cannot directly model. Consequently, we cannot draw straightforward conclusions about true indoor versus outdoor preferences. Still, assuming that these biases affect both species similarly, the comparison between them may remain reliable.

To address these biases, we modelled the relative indoor frequency of *Ae. albopictus* relative to *Cx. pipiens* across the study period. Results from MODEL-2 showed that indoors *Ae. albopictus* is more frequently reported than *Cx. pipiens* from late June to mid-October (i.e. >60%), reaching 77% in mid-August. This pattern may reflect the effect of the two species population dynamics in southern Europe, where *Ae. albopictus* peaks between late July to October, while *Cx. pipiens* is most abundant in colder spring and autumn months (Da Re et al., 2025; Petrić et al., 2014; Veronesi et al., 2012). Nevertheless, the pronounced curvilinear trend suggests that the

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probability of *Ae. albopictus* indoor presence during its peak activity period is higher than for *Cx. pipiens*.

Results of a preliminary entomological assessment carried out at small scale in Italy to compare the fraction of *Ae. albopictus* population collected by conventional entomological traps located indoors and outdoors are consistent with citizen science-based results. In the first 2 weeks of September (when most specimens were collected), the mean daily proportion of female *Ae. albopictus* captured indoors versus outdoors ranged from 19% to 55% in Rome and from 26% to 44% in Padua. These observations confirm that *Ae. albopictus* may successfully exploit indoor environments, corroborating citizen records and the relevance of indoor exposure for human–mosquito interactions.

4.1 | Limitation of the study

As implicit in citizen science-based studies, the data here analysed derive from volunteer-submitted observations which allow spatial and temporal coverage not achievable by conventional approaches, but present inherent limitations. First, the number of records across years and regions is strongly affected by the capacity to involve citizens in the project (Virgillito et al., 2024). The communication campaigns implemented in Italy and Spain differed in means and goals in relation to different timing in Mosquito Alert implementation (since 2014 in Spain and since end of 2020 in Italy), as well as to *Ae. albopictus* invasion history in the two countries (2004 in Spain, Aranda et al., 2006; 1990 in Italy; Sabatini et al., 1990). However, this limitation is not crucial for the purpose of the present study, which is not meant to identify differences between countries and among years, but only to provide evidence of indoor presence not detectable otherwise.

A second limitation is that citizen science data are hard to be validated. In this study, only Mosquito Alert photographic records of *Ae. albopictus* and *Cx. pipiens* validated by the Entolab experts (MA EntoLab Terms of Use—www.mosquitoalert.com) were included. However, whether the specimen was collected indoors or outdoors prior to the photograph being taken was not verifiable, and we therefore relied on the answer provided by users through the mandatory selection step during the record submission process. Moreover, the personal criteria to classify an indoor or outdoor environment (e.g. how is a patio classified?) constitute an additional source of bias. Despite the lack of validation of this information crucial for the present study, although some misclassifications may have occurred, there is no reason to believe that the majority of the reports were not correctly classified. This is also supported by results by Virgillito et al. (2024) showing a high performance of citizen scientists in mosquito species/genus identification.

A third, more relevant limitation of the study is the assumption that the users' capacity to collect/photograph a mosquito specimen does not differ for the two species, nor between the indoor and outdoor environment. Speculations based on results obtained are likely biased by an underestimation of *Culex* records due to either a higher difficulty in spotting the species during its night-biting activity (Padilla-Pozo et al., 2024) and/or because users' participation is suspended during sleeping hours (Pernat et al., 2021). However, blood-fed *Culex* females are easy to spot on household walls and to photograph in the morning. On the other hand, *Ae. albopictus* is day-active and thus interacts with vigilant people, but may be more difficult to collect/photograph due to its evasive biting behaviour and its resting phase on outdoor vegetation (Dalpadado et al., 2022; Samson et al., 2013). Moreover, it can be claimed that citizens' propensity to engage in the different steps of the submission process may be higher indoors than outdoors, leading to overestimations of indoor records. This latter bias was overcome in MODEL-2 which is focused on indoor records only, allowing for a refined analysis of indoor species composition. While we cannot exclude these sources of bias, the convergence of multiple validation checks (see Section 2) and the consistent patterns resulting from the two models reinforce confidence in our central conclusion of a not negligible presence of *Ae. albopictus* indoors.

Finally, in addition to the citizen science-based limitations, it is important to note that all Mosquito Alert photographic records of *Culex* spp. were included in the dataset and discussed in the text as *Cx. pipiens*. Even though this is the dominant species of the genus in Europe (Becker et al., 2010; Di Luca et al., 2016; Haba & McBride, 2022; Widlake et al., 2025) it belongs to cryptic morphological undistinguishable species (Alout et al., 2025; Harbach, 2012). To address this possible bias, we molecularly identified a subsample of 329 Culex spp. specimens mailed by citizen in Italy to Sapienza University and recorder in MA, and all of these were identified as *Cx. pipiens*. Moreover, *Cx. pipiens* in Europe is known to include two morphologically indistinguishable ecotypes, the endophagic and anthropophagic *Cx. p. molestus* and the exophagic and ornithophagic *Cx. p. pipiens* (Harbach, 2012). *Culex p. molestus*/*Cx. p. pipiens* hybrids are known to be frequent in Italy and to be characterised by biting behaviours intermediate between the two ecotypes (Di Luca et al., 2016). Molecular identification of the above mentioned 329 specimens showed that 62%, 18% and 20% of these were *Cx. p. molestus*, hybrids and *Cx. p. pipiens*, respecively (File S8).

5 | CONCLUSIONS

The results clearly highlight an unexpected level of *Ae. albopictus* indoor presence in southern Europe. About 50% of *Ae. albopictus* Mosquito Alert photographic records are taken indoors from June to October and approximately six out of ten photos taken by users indoors are of *Ae. albopictus*. The results also highlight the great potential of citizen science to provide large-scale evidence and reveal animal behavioural patterns whose assessment by conventional approaches is unfeasible. This case study is paradigmatic (particularly in temperate countries) as the lack of indoor sampling in regular mosquito monitoring schemes may conceal a public health relevant behaviour.

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Larger efforts are needed to quantify *Ae. albopictus* actual degree of endophagy/endophily in its invasive range. Nevertheless, the sole evidence of relevant *Ae. albopictus* indoor presence is epidemiologically valuable. If we assume that most females enter the houses looking for a host—as supported by the results of the field experiment showing that about a third of host-seeking *Ae. albopictus* females were collected indoors—this would imply that in the event of outbreaks of *Ae. albopictus*-borne arboviruses, indoor transmission may not be negligible. This is also supported by studies on transmission chains of Dengue and Chikungunya showing that human cases are often concentrated among individuals within the same household (Bloch et al., 2016; Guzzetta et al., 2020; Martínez-Vega et al., 2015; Molina Grané et al., 2026; Ribeiro dos Santos et al., 2022; Salje et al., 2016). In turn, this implies that European and national guidelines for exotic arbovirus prevention and control should put more emphasis on risk of indoor transmission and in recommending indoor protective measures (e.g. window nets/screens, environmental repellents). This is particularly significant in consideration of the raising number of autochthonous dengue and chikungunya cases reported in Europe in the last years (Cattaneo et al., 2025; www.ecdc.europa.eu) confirming models' expectations warning about the future possible establishment of these diseases in temperate regions (Farooq et al., 2025; Higgs & Vanlandingham, 2015; Laverdeur et al., 2024; Menegale et al., 2025; Parreira & Sousa, 2015; Zardini et al., 2024).

AUTHOR CONTRIBUTIONS

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Some of the data that support the findings of this study are available on request from the corresponding authors (Eleonora Longo and Beniamino Caputo). The data are not publicly available due to their containing information that could compromise the privacy of research participants. However, the open data used in this analysis are available in Zenodo at https://doi.org/10.5281/zenodo.597466 (Escobar Rubies & Mosquito Alert, 2026) and https://doi.org/10.5281/zenodo.5802476 (Mosquito Alert, 2026) and are documented in the Mosquito Alert Data Portal at https://labs.mosquitoalert.com/metadata_public_portal/README.html.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Figure S1. Number of validated records of *Ae. albopictus* (Left) and *Cx. pipiens* (Right) over biweekly periods in Italy and Spain.

Figure S2. SE outliers' threshold.

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Figure S3. Relationship between observed number of MA records and SE (expected records) across spatial cells.

Figure S4. Seasonal observed indoor rates of *Ae. albopictus* (red) and *Cx. pipiens* (blue) MA photographic records from Italy and Spain (April to November 2021–2024).

Figure S5. Daily female *Ae. albopictus* indoor ratio captured indoors in Rome (Lazio) and Padua (Veneto) during late summer 2024 (26 August–06 September).

Table S1. Comparison of MODEL-1 and MODEL-2 performance with and without SE outliers.

Table S2. Modified DB for MODEL-1 and MODEL-1best analyses.

Table S3. Modified DB for MODEL-2 and MODEL-2best analyses.

Table S4. Model selection results for MODEL-1.

Table S5. Model selection results for MODEL-2.

Table S6. Summary of species-specific indoor versus outdoor nuisance model (MODEL-1 best).

Table S7. Summary of indoor *Ae. albopictus* versus *Cx. pipiens* nuisance model (MODEL-2 best).

File S1. Mosquito Alert app description and photographic mosquito records validation process.

File S2. SE outliers' removal.

File S3. Sensitivity analysis of SE outliers' removal.

File S4. Validation checks to ensure reliability of spatial and temporal metadata in DB and preliminary statistical analysis to evaluate year effect.

File S5. Modification and Standardisation of the DB dataset for MODEL-1 and MODEL-1best analyses.

File S6. Modification and Standardisation of the DB dataset for MODEL-2 and MODEL-2best analyses.

File S7. Geographical distribution of records.

File S8. Citizen-based mosquito sampling and species identification methods.

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