PLOS ONE

Mitigating the threat of invasive mosquito species expansion: a comprehensive entomological surveillance study on Kastellorizo, a remote Greek island

--Manuscript Draft--

Manuscript Number:	PONE-D-23-43125
Article Type:	Research Article
Full Title:	Mitigating the threat of invasive mosquito species expansion: a comprehensive entomological surveillance study on Kastellorizo, a remote Greek island
Short Title:	Short mission in a remote island in Greece: first record of Aedes albopictus in Kastellorizo
Corresponding Author:	Antonios Michaelakis Benaki Phytopathological Institute Kifisia-Athens, GREECE
Keywords:	Aedes albopictus; Aedes cretinus; KAP questionnaire; vectors
Abstract:	The expansion of the tiger mosquito, a vector that can transmit diseases such as dengue, chikungunya, and Zika virus, poses a growing threat to global health. This study focuses on the entomological surveillance of Kastellorizo, a remote Greek island affected by its expansion. The research employs a multifaceted approach, combining public questionnaires, mosquito collection using adult traps and human landing catches, and morphological and molecular identification methods. Results from questionnaires reveal community awareness and preparedness gaps, emphasizing the need for targeted education. Mosquito collections confirm the presence of the Aedes albopictus, Aedes cretinus and Culex pipiens mosquitoes, highlighting the importance of surveillance. The study underscores the significance of community engagement in entomological efforts and proposes a citizen science initiative for sustained monitoring. Overall, the research provides essential insights for developing effective mosquito control programs in remote island settings.
Order of Authors:	Marina Bisia
	Georgios Balatsos
	Stavroula Beleri
	Nikolaos Tegos
	Evangelia Zavitsanou
	Shannon L LaDeau
	Vasilis Sotiroudas
	Elina Patsoula
	Antonios Michaelakis
Opposed Reviewers:	
Additional Information:	
Question	**Response**
Financial Disclosure Enter a financial disclosure statement that describes the sources of funding for the work included in this submission. Review the submission guidelines for detailed requirements. View published research articles from *PLOS ONE* for specific examples.	Yes

This statement is required for submission and **will appear in the published article** if the submission is accepted. Please make sure it is accurate. **Funded studies** Enter a statement with the following details: • Initials of the authors who received each award • Grant numbers awarded to each author • The full name of each funder • URL of each funder website • Did the sponsors or funders play any role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript? Did you receive funding for this work?	
Please add funding details. as follow-up to "**Financial Disclosure** Enter a financial disclosure statement that describes the sources of funding for the work included in this submission. Review the submission guidelines for detailed requirements. View published research articles from *PLOS ONE* for specific examples. This statement is required for submission and **will appear in the published article** if the submission is accepted. Please make sure it is accurate. **Funded studies** Enter a statement with the following details: • Initials of the authors who received each award • Grant numbers awarded to each author • The full name of each funder • URL of each funder website • Did the sponsors or funders play any role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript?	This article is based upon work within the framework of the Horizon Europe program through the project IDAlert (https://idalertproject.eu) with grant no. 101057554. IDAlert is part of the EU climate change and health cluster (https://climate-health.eu). It was also supported by the project “A systematic surveillance of vector mosquitoes for the control of mosquito-borne diseases in the Region of Attica", financed by the Region of Attica. The funders had no role in study design, data collection and analysis, the decision to publish, or the preparation of the manuscript.

Did you receive funding for this work?"	
Please select the country of your main research funder (please select carefully as in some cases this is used in fee calculation). as follow-up to "**Financial Disclosure** Enter a financial disclosure statement that describes the sources of funding for the work included in this submission. Review the submission guidelines for detailed requirements. View published research articles from *PLOS ONE* for specific examples. This statement is required for submission and **will appear in the published article** if the submission is accepted. Please make sure it is accurate. **Funded studies** Enter a statement with the following details: • Initials of the authors who received each award • Grant numbers awarded to each author • The full name of each funder • URL of each funder website • Did the sponsors or funders play any role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript? Did you receive funding for this work?"	GREECE - GR
Competing Interests Use the instructions below to enter a competing interest statement for this submission. On behalf of all authors, disclose any competing interests that could be perceived to bias this work—acknowledging all financial support and any other relevant financial or non-financial competing interests. This statement is required for submission	The authors have declared that no competing interests exist

and **will appear in the published article** if the submission is accepted. Please make sure it is accurate and that any funding sources listed in your Funding Information later in the submission form are also declared in your Financial Disclosure statement. View published research articles from *PLOS ONE* for specific examples. **NO authors have competing interests** Enter: *The authors have declared that no competing interests exist.* **Authors with competing interests** Enter competing interest details beginning with this statement: *I have read the journal's policy and the authors of this manuscript have the following competing interests: [insert competing interests here]* * typeset	
Ethics Statement Enter an ethics statement for this submission. This statement is required if the study involved: • Human participants • Human specimens or tissue • Vertebrate animals or cephalopods • Vertebrate embryos or tissues • Field research Write "N/A" if the submission does not require an ethics statement. General guidance is provided below. Consult the submission guidelines for detailed instructions. **Make sure that all information entered here is included in the Methods section of the manuscript.**	The current study did not involve endangered or protected species and KAP (knowledge, attitudes and practices) study does not reveal confidential information regarding human participants. The current study was implemented in accordance with the Ethics Code for Research of Benaki Phytopathological Institute.

Format for specific study types **Human Subject Research (involving human participants and/or tissue)** • Give the name of the institutional review board or ethics committee that approved the study • Include the approval number and/or a statement indicating approval of this research • Indicate the form of consent obtained (written/oral) or the reason that consent was not obtained (e.g. the data were analyzed anonymously) **Animal Research (involving vertebrate animals, embryos or tissues)** • Provide the name of the Institutional Animal Care and Use Committee (IACUC) or other relevant ethics board that reviewed the study protocol, and indicate whether they approved this research or granted a formal waiver of ethical approval • Include an approval number if one was obtained • If the study involved *non-human primates*, add *additional details* about animal welfare and steps taken to ameliorate suffering • If anesthesia, euthanasia, or any kind of animal sacrifice is part of the study, include briefly which substances and/or methods were applied **Field Research** Include the following details if this study involves the collection of plant, animal, or other materials from a natural setting: • Field permit number • Name of the institution or relevant body that granted permission	
Data Availability Authors are required to make all data underlying the findings described fully available, without restriction, and from the time of publication. PLOS allows rare exceptions to address legal and ethical concerns. See the PLOS Data Policy and FAQ for detailed information.	Yes - all data are fully available without restriction

A Data Availability Statement describing where the data can be found is required at submission. Your answers to this question constitute the Data Availability Statement and **will be published in the article**, if accepted. **Important:** Stating 'data available on request from the author' is not sufficient. If your data are only available upon request, select 'No' for the first question and explain your exceptional situation in the text box. Do the authors confirm that all data underlying the findings described in their manuscript are fully available without restriction?	
Describe where the data may be found in full sentences. If you are copying our sample text, replace any instances of XXX with the appropriate details. • If the data are **held or will be held in a public repository**, include URLs, accession numbers or DOIs. If this information will only be available after acceptance, indicate this by ticking the box below. For example: *All XXX files are available from the XXX database (accession number(s) XXX, XXX.*). • If the data are all contained **within the manuscript and/or Supporting Information files**, enter the following: *All relevant data are within the manuscript and its Supporting Information files.* • If neither of these applies but you are able to provide **details of access elsewhere**, with or without limitations, please do so. For example: *Data cannot be shared publicly because of [XXX]. Data are available from the XXX Institutional Data Access / Ethics Committee (contact via XXX) for researchers who meet the criteria for access to confidential data.* *The data underlying the results presented in the study are available from (include the name of the third party*	All relevant data are within the manuscript and its Supporting Information files.

and contact information or URL). • This text is appropriate if the data are owned by a third party and authors do not have permission to share the data. * typeset	
Additional data availability information:	Tick here if the URLs/accession numbers/DOIs will be available only after acceptance of the manuscript for publication so that we can ensure their inclusion before publication.

December 21, 2023

Dear Editor,

I am writing to submit our paper titled "Mitigating the threat of invasive mosquito species expansion: a comprehensive entomological surveillance study on Kastellorizo, a remote Greek island" to be considered for publication in PLOS ONE.

Our study employs a comprehensive approach, utilizing public questionnaires, mosquito collection through various methods, and morphological and molecular identification techniques. The findings underscore critical gaps in community awareness and preparedness, highlighting the urgent need for targeted education. The confirmation of *Aedes albopictus*, *Aedes cretinus*, and *Culex pipiens* presence emphasizes the importance of robust surveillance efforts. Additionally, the study advocates for community engagement and proposes a citizen science initiative for sustained monitoring.

We firmly believe that our study's findings provide essential insights for developing effective mosquito control programs in remote island settings. Furthermore, I believe that this study contributes significantly to the understanding of vector-borne disease risks in remote areas.

Considering the esteemed reputation of PLOS ONE, we are confident that your journal is the ideal platform to showcase our work. We appreciate your time and consideration in reviewing our submission.

Thank you for your attention.

Yours sincerely,

Dr Antonios Michaelakis

Laboratory of Insects & Parasites of Medical Importance

Benaki Phytopathological Institute

Mitigating the threat of invasive mosquito species expansion: a comprehensive entomological surveillance study on Kastellorizo, a remote Greek island.

Marina Bisia[1¶], Georgios Balatsos[1¶], Stavroula Beleri[2], Nikolaos Tegos[2], Evangelia Zavitsanou[1], Shannon L. LaDeau[3], Vasilis Sotiroudas[4], Elina Patsoula[2], Antonios Michaelakis[1*]

[1]Laboratory of Insects and Parasites of Medical Importance, Scientific Directorate of Entomology and Agricultural Zoology, Benaki Phytopathological Institute, 14561 Kifissia, Greece.

[2]Department of Public Health Policy, Laboratory for Surveillance of Infectious Diseases-Unit of Medical Entomology, School of Public Health, University of West Attica, 11521 Athens, Greece.

[3]Cary Institute of Ecosystem Studies, Millbrook, NY 12545, USA.

[4]AgroSpeCom, 7th klm national road Thessaloniki-Katerini, Kalochori, 57009, Thessaloniki, Greece

*Corresponding author

Email: a.michaelakis@bpi.gr

¶ These authors contributed equally to this work

Abstract

The expansion of the tiger mosquito, a vector that can transmit diseases such as dengue, chikungunya, and Zika virus, poses a growing threat to global health. This study focuses on the entomological surveillance of Kastellorizo, a remote Greek island affected by its expansion. The research

employs a multifaceted approach, combining public questionnaires, mosquito collection using adult traps and human landing catches, and morphological and molecular identification methods. Results from questionnaires reveal community awareness and preparedness gaps, emphasizing the need for targeted education. Mosquito collections confirm the presence of the *Aedes albopictus, Aedes cretinus* and *Culex pipiens* mosquitoes, highlighting the importance of surveillance. The study underscores the significance of community engagement in entomological efforts and proposes a citizen science initiative for sustained monitoring. Overall, the research provides essential insights for developing effective mosquito control programs in remote island settings.

Introduction

As stated in the "Global Vector Control Response (GVCR) 2017–2030", a big challenge for all societies is "the growing burden and threat of vector-borne diseases to human health" (1). Changing climate and global connectivity are major drivers of the changing patterns of vector-borne diseases as both alter the distribution, abundance and behavior of arthropod vectors and the pathogens they carry (2). The mosquito- vector is implicated in the transmission of several diseases that are spreading to new areas, including dengue fever, chikungunya, and the Zika virus (3–6). Likewise, the success story of the expansion of *Aedes (Ae.) albopictus* in many parts of the world raises the alarm for these diseases in regions where they were previously absent (7,8).

Monitoring mosquito-borne disease risk in rural and island communities presents unique challenges (9). The rapid spread of the two globally concerning *Aedes* species, *Ae. aegypti* and *Ae. albopictus*, is reshaping human risk in ways that remain poorly understood (10). While considerable

attention has been directed towards large urban centers, the dynamics of how these mosquito populations establish and proliferate in smaller towns and villages, which are increasingly interconnected through global trade and travel, remain understudied (11). Entomological surveillance is an important tool for identifying new foci of disease risk (12). By collecting data on mosquito abundance, species composition, and infection rates, valuable information can be provided in order to implement effective mosquito control programs (13). Keeping track of *Ae. albopictus* mosquito population through entomological surveillance is essential to monitor their spread and prevent transmission of diseases (14–16). Entomological surveillance can, however, be expensive and time intensive, especially when little is known apriori about species composition or locations of mosquito breeding habitat. Several studies have demonstrated that public questionnaires can provide useful information about perceived mosquito nuisance and resident-led control actions (17–19). Similar survey methods may be effective for identifying important characteristics of mosquito exposure in new locations.

Greece is one of the European countries that has been affected by the expansion of *Ae. albopictus* in recent years (20,21). The mosquito was first detected between 2003 and 2004 and has since spread to many parts of the country, including the Aegean islands (22). Several studies have reported the establishment and spread of this mosquito species, which highlights the need for strengthening entomological surveillance (23,24).

Greece's unique geography, with 114 inhabited islands (25), makes it challenging to conduct entomological surveillance due to limited physical access, equipment, and resources. The available data on entomological surveillance and control programs for islands is significantly lacking. As of 2023, only about one third of these islands, primarily the larger ones with accessible air and marine transportation, have implemented such programs. This leaves a substantial portion of islands without any documented information on these crucial efforts. The absence of entomological surveillance initiatives on small islands warrants attention and resolution. To address this issue, we developed a protocol to assess the extent of

knowledge acquisition within a limited duration, emphasizing the efficiency of brief visits for data gathering in remote areas, such as the island of Kastellorizo in Greece.

So, in this study, our focus is on evaluating the comprehensive insights provided by different types of information collected, contributing to a holistic synthesis of mosquito exposure, disease risk assessment, and potential control actions. The protocol involves a) questionnaires to the public conducted through a door-to-door approach; b) collection of mosquitoes using adult traps and ovitraps, providing data on both adult mosquito populations and their breeding sites; and c) collecting of mosquitoes through human landing catches (a method involving the direct interaction with humans acting as bait), offering insights into the mosquitoes' host-seeking behavior. By employing these varied approaches, we aim to enhance our understanding of the dynamics between mosquito populations, human exposure, and the associated disease risks, ultimately informing targeted and effective control measures.

Materials and Methods

Study site

Kastellorizo (or Megisti) is a small Greek island located in the southeastern Aegean Sea. It is the easternmost inhabited island of Greece and is situated roughly 2 kilometers (1.2 miles) off the south coast of Turkey. The island's port operates daily, with two weekly departures specifically designated for voyages to Turkey, particularly during the summer months. The island has a total area of about 9 square kilometers (3.5 square miles) and a population of around 500 people, according to the most recent data available (25). In 2023, the local municipal authorities recognized the necessity for a mosquito control program primarily driven by public nuisance complaints, particularly during the high tourist season. Importantly,

there have been no recorded cases of vector-borne diseases on the current island. The application of the control program is focused on the management of public breeding sites but with no existing baseline data on local species composition and mosquito abundance.

Questionnaire

The questionnaire procedure used in this study was based on previously established protocols and methods used in similar projects (19,26,27). These protocols were carefully selected and adapted to the specific context and goals of our study (Annex questionnaire 1). In our study, we employed a random sampling technique to ensure representative participation. Specifically, we targeted permanent residents who frequented the coastal area during the morning hours, when various establishments like restaurants, cafes, banks, and supermarkets were bustling with activity. In selecting the singular location for questionnaire distribution, it is noteworthy that this specific site stands as the sole hub on the island boasting a diverse array of establishments. The unique concentration of these amenities in this particular area ensures that the survey reaches a broad cross-section of the island's population. This approach allowed us to capture a diverse cross-section of the community for our questionnaires, enhancing the robustness of our data analysis. The primary objectives of our questionnaire encompass three key goals. Firstly, it aimed to identify the presence and prevalence of mosquitoes on the island. Secondly, it sought to categorize mosquito species based on their biting behavior and preferred habitats, as different species may require distinct approaches. This information is crucial for tailoring effective control strategies. Lastly, the questionnaire aimed to evaluate the community's readiness to adopt and implement mosquito control measures. This is vital, especially in an event of an emergency health linked to mosquitoes, as it provides insights into their knowledge and capacity to respond effectively.

Mosquito Collection

We employed three methods for collecting mosquito specimens that each target a specific life or activity stage of juvenile and adult mosquito species likely to bite humans. The specific protocols in this study targeted *Culex* and *Aedes* species which are the main mosquito species in urban and suburban areas in Greece (28,29).

Oviposition Traps

The selection of an entomological surveillance method is based on clear objectives, available resources, and disease prevalence. Standardization enhances data quality and enables seamless sharing and analysis (30,31). In the case of the remote island of Kastellorizo, employing ovitraps proves promising, as it is a low-cost method and requires little expertise or training to implement. Supported by established SOPs, citizen engagement through the Mosquito Alert App, and comprehensive training resources, this approach offers an effective means to monitor and manage mosquito populations, crucial for public health (32). In this study, from 7 to 26 July 2022, five oviposition traps (plastic cups containing Xml water and a wooden tongue depressor) were deployed in areas with partial or full shading, with a maximum height of 50 cm from the ground. The positioning of these traps aimed to cover the widest possible area and to be in close proximity to potential mosquito breeding grounds, such as water pools and used vehicle tires left outdoors, as well as resting places such as trees and bushes. Each tongue depressor used as an oviposition substrate was marked with a unique code for identification. During collection, the tongue depressors were wrapped in damp gauze to prevent the eggs from dehydrating and were stored in transparent plastic sampling bags labeled with information about the sample, date of collection, and location (32).

Adult Traps

To better understand the distribution and prevalence of mosquito species on the island, 4 BG-Sentinel type traps were placed in different areas. The municipality provided assistance in areas where

residents had raised concerns regarding mosquito nuisance. These traps were operational for a period of 2 days (from 25 to 27 July 2022), equipped with an attractant that mimics the odor of human skin by gradually releasing chemical compounds that are attractive to mosquitoes. Each trap, powered by electricity, was strategically positioned in secure and private locations, effectively covering an area of up to 4 acres. Once the mosquitoes were caught in the trap, they were collected and stored on ice to preserve their condition for later analysis. The use of attractants in traps has been shown to be an effective method for monitoring mosquito populations and studying their behavior (28,29).

Human Landing Catches

A human baiting method (HLC) was also employed to collect adult mosquitoes. The task was assigned to a member of the team that approached the habitat, with potential resting places for the mosquitoes, and allowed them to land on their body, and then used an electric aspirator to capture them. The collection was conducted during the late afternoon hours (from 6:00 pm to 7:00 pm). A total of 9 different points were selected for the sampling process, and two samplings (for 2 consecutive days) were carried out per point. This method was used to collect samples from different natural or artificial resting places of adult mosquitoes, including water collections, bushes, and underground areas.

The collected samples were preserved by storing them at -20°C until their transportation to the laboratory of Insects and Parasites of Medical Importance at the Benaki Phytopathological Institute for further studies. In the lab, they were carefully examined to ensure the accurate morphological identification (33,34). In addition, selected samples were transferred to the Laboratory of Medical Entomology, of Public Health Policy at the University of West Attica, where molecular techniques were utilized to aid in the identification of samples. This approach was particularly useful for samples that were damaged or difficult to identify based on their morphology alone.

Molecular procedure

DNA Extraction and Polymerase Chain Reaction (PCR) Amplification.

A total of 33 adult mosquitoes that were morphologically identified were also examined at the molecular level to verify species identification. DNA was extracted from individual whole adults, using the NucleoSpin Tissue, DNA Mini kit (MACHEREY-NAGEL, Germany), following manufacturers' instructions. The nuclear ribosomal spacer gene ITS2 was amplifed by PCR using 5,8S and 28S primers and a part of the mitochondrial cytochrome oxidase I gene (COI) was also amplifed using primers C1-J-1718 and C1-N-2191, with related PCR protocols being carried out as previously described (35,36)

Products were electrophoresed and sent for sequencing analysis (CEMIA, SA, Greece). Similarity with sequences available in GenBank was assessed using the Basic Local Alignment Tool (BLAST), Blastn, and sequences were aligned using the CLUSTAL omega software (EMBL-EBI).

Results

Questionnaires

As previously mentioned, the primary goal of this study is to evaluate the importance of diverse information sources in constructing a comprehensive synthesis of mosquito exposure, disease risk, and potential control strategies. In specific reference to the questionnaires, our main aim was to assess the magnitude of the human exposure to mosquitoes on the island. This objective was addressed through a targeted analysis of questions 1, 3, 9, and 14 within the questionnaire framework. Out of the total participants, 51.61% reported experiencing a significant exposure to mosquitoes, while 29.03% indicated

a considerable presence, and 19.35% reported a high number of mosquitoes in their area. The overall level of annoyance caused by mosquitoes was notably high, with 66.67% of participants expressing extreme annoyance, and 20% reporting a great degree of irritation. Nearly all participants (90.32%) reported taking measures to protect themselves and their families from mosquitoes. However, a majority (70%) stated that these protective measures were insufficient and not very effective.

Our second objective was to classify mosquito species based on their biting habits and preferred habitats. Analysis of the questionnaire yielded the following insights: A significant majority of respondents (80.77%) noted mosquito activity primarily during the night, while 19% reported sightings during both day and night. In terms of seasonal patterns, 46.15% of participants indicated the onset of mosquito issues necessitating personal protective equipment as early as January. The remaining respondents reported this starting between April and July. Furthermore, 42.31% of participants identified December as the month when mosquito problems typically subside, suggesting an almost year-round presence of mosquitoes.

The third goal centered on assessing the community's preparedness. It was observed that 67.74% of participants provided incorrect answers regarding mosquito breeding sites, while only 32.26% accurately identified the correct locations. Similarly, in terms of which mosquitoes bite (male, female, or both), 56.67% of participants provided incorrect responses, while only 43.33% gave the correct answer (female). Notably, the Asian mosquito tiger was correctly identified as a new, non-local species by a significant majority (96.55%) of participants. When presented with images, only 13 (44.83%) participants were able to correctly identify the mosquito tiger. Additionally, the data indicated that 53.85% of participants accurately recognized the actual threatening insects by selecting the images of mosquitoes. Regarding protective measures, a majority of participants (61.29%) stated that they frequently utilized natural methods, while 35.48% reported using chemical measures often. Financially, the majority (73.91%) reported allocating 20€ or more per month for personal protection, which notably exceeds the minimum wage set at 780€. Opinions regarding the effectiveness of mosquito population control methods were

divided. Specifically, 20 (64.52%) participants reported that both natural and chemical methods were effective, while 8 (25.81%) and 3 (9.68%) participants favored chemical and natural methods, respectively. The initial three questions were also used to provide the research team with preliminary insights into the mosquito species present in the study area.

In terms of demographics, most participants (93.55%) reported living on Kastellorizo permanently, and there were more female participants (64.52%). The demographic information for the questionnaire responses is shown in Table 1.

Table 1. Questionnaire responses (*n*=31) on demographics

Demographic Information	**Number of responses (%)**
Sex	
Woman	20 (64,52)
Man	11 (35,48)
Type of settlement	
Permanent residence	29 (93,55)
Holiday residence	2 (6,45)

Mosquito Collections

The data obtained from the ovitraps collections revealed that there is a well-established population of the *Ae. albopictus* present. In total 203 eggs were collected. Detailed results of the egg

collection from the traps, as well as their exact locations, are provided in Table 2. Fig 1 illustrates the distinct placement of the collection methods utilized, including ovitraps, BG traps, and HLC. Additionally, it provides an overview of the mosquito species that were collected.

Table 2. Information regarding the ovitrap collections in the 5 different locations in the island of Kastellorizo.

Ovitraps	*Lat*	*Long*	*Installation day*	*Collection day*	*No. of eggs*
K1	36.149806°	29.592298°	12/07/22	26/7/22	2
K2	36.149044°	29.589423°	12/07/22	26/7/22	32
K3	36.148920°	29.589885°	12/07/22	26/7/22	3
K4	36.148889°	29.590789°	12/07/22	26/7/22	2
K5	36.148671°	29.591501°	12/07/22	26/7/22	164

Fig 1. The sites of the three collection methods and the mosquito species that were collected.

The morphological identification (34) of the mosquitoes collected from the adult traps showed the presence of two mosquito species - the Asian tiger mosquito (*Ae. albopictus*) and the common mosquito {*Culex (Cx.) pipiens*} (Table 2). In total 79 mosquitoes (73 ♀ and 6 ♂) were *Cx. pipiens*, while only 1♀ belonged to *Ae. albopictus* species. The common mosquito was found to be the dominant species in adult trap collections. A static map was then created to show a comparative display of the results (Fig 2).

Table 3. Information regarding the adult collections in the 4 different locations on the island of Kastellorizo.

Adult traps	*Location*	*Lat*	*Long*	*Installation day*	*Collection day*	*Cx. pipiens*		*Ae. albopictus*	
						♀	♂	♀	♂
BG1	Airport	36.142480°	29.576363°	25/07/2022	27/07/2022	0	1	0	0
BG2	Army infrastructure	36.149609°	29.584378°	25/07/2022	27/07/2022	41	2	0	0
BG3	Mandraki	36.149468°	29.597007°	25/07/2022	27/07/2022	4	0	0	0
BG4	Square (Lazarakis)	36.148968°	29.590747°	25/07/2022	27/07/2022	28	3	1	0

Fig 2. Results of the *Cx. pipiens* females for the 4 different locations in the island of Kastellorizo (Megisti).

To collect mosquitoes using the method of human landing catches, 9 specific locations were selected, as indicated in Table 3. The results revealed the high activity of the Asian tiger mosquito. In total 23 female mosquitoes and 12 male mosquitoes were collected belonging to *Ae. albopictus* species whereas 3 (1 ♀ and 2 ♂) mosquitoes were *Cx pipiens*. Fig 3 displays a static map, showing a comparative view of the results from the human landing collection of female *Ae. albopictus* mosquitoes.

Table 4. Table indicating the locations where adult mosquitoes were collected using the human landing collection method and the recorded species identification results.

HLC	*Lat*	*Long*	*Collection day*	*Cx. pipiens*		*Ae. albopictus*	
				♀	♂	♀	♂
K_HLC1	36.149822°	29.588870°	25/7/2022	0	0	1	1
K_HLC2	36.149230°	29.589221°	25/7/2022	1	2	2	3
K_HLC3	36.148804°	29.589596°	25/7/2022	0	0	0	0
K_HLC4	36.148840°	29.590294°	25/7/2022	0	0	0	0
K_HLC5	36.148499°	29.591061°	25/7/2022	0	0	0	0
K_HLC6	36.148679°	29.591763°	25/7/2022	0	0	0	0
K_HLC7	36.148969°	29.592251°	25/7/2022	0	0	0	0
K_HLC8	36.149701°	29.592360°	25/7/2022	0	0	0	0
K_HLC9	36.150406°	29.592660°	25/7/2022	0	0	0	0
K_HLC1	36.149822°	29.588870°	26/7/2022	0	0	0	0
K_HLC2	36.149230°	29.589221°	26/7/2022	0	0	3	1
K_HLC3	36.148804°	29.589596°	26/7/2022	0	0	9	6

K_HLC4	36.148840°	29.590294°	26/7/2022	0	0	3	0
K_HLC5	36.148499°	29.591061°	26/7/2022	0	0	2	0
K_HLC6	36.148679°	29.591763°	26/7/2022	0	0	1	1
K_HLC7	36.148969°	29.592251°	26/7/2022	0	0	2	0
K_HLC8	36.149701°	29.592360°	26/7/2022	0	0	0	0
K_HLC9	36.150406°	29.592660°	26/7/2022	0	0	0	0

Fig 3. Static map of the results of the *Ae. albopictus* females for the 9 different locations in the island of Kastellorizo (Megisti).

Molecular procedures

In order to ensure the morphological identification, a subset of samples was randomly selected for molecular identification. Additionally, molecular procedures were employed for several mosquito samples that exhibited a loss of identifiable characteristics, preventing their morphological identification. Around 41% of the total number of the mosquitoes were tested with molecular procedures, using two different amplification protocols. These approaches were undertaken to enhance the reliability and robustness of the identification process. Amplification by PCR of ITS and COI fragments produced the expected fragments of *Ae. albopictus and Cx. pipiens.* A very interesting result was the *Ae. cretinus* sample

253 that was identified after further analysis of the sequencing chromatograms, thus verifying the presence of
254 both *Aedes* species in the island.

255

256

257 Discussion

258 Of the 60 species of mosquitoes that have been recorded in Greece (34,37,38), 3 were identified
259 in Kastellorizo in this study. Traps were placed in several sites, representing different types of environments
260 and although the study was of short scale, findings are noteworthy, since species of public health
261 importance, such as *Ae. albopictus*, a species with worldwide distribution, potential vector of Dengue,
262 Chikungunya and Zika, and *Cx. pipiens*, important vector of West Nile virus (WNV) and other pathogens,
263 were detected. *Cx. pipiens* was the most prevalent species in this study and is related with outbreaks of
264 West Nile fever that are annually reported in several European countries.

265 In Greece *Ae. albopictus* and *Ae. cretinus* have been recorded together since 2003 and are known
266 to be closely related species with common morphological features and ecological similarities
267 (20,22,27,36). It is not of surprise that *Ae. albopictus* is also present in this remote island given its known
268 ability to adapt in most environmental types and its spread and establishment in Europe do far. As it is
269 involved in pathogen transmission, surveillance and control is of major importance (32).

270 The recent introduction of *Ae. aegypti* in Cyprus ref highlights the need for comprehensive
271 entomological surveillance not only on the island but also in the surrounding remote areas (39).
272 Mosquitoes are known to travel great distances, and as such, the introduction of *Ae. aegypti* to Cyprus
273 may have occurred due to the movement of people or goods from neighboring countries (40). Monitoring

remote areas, especially those that share borders with countries where *Ae. aegypti* is endemic, is crucial to detect the presence of the mosquito early on and prevent its establishment in new areas (41).

Entomological surveillance is especially critical in remote islands where the risk of vector-borne diseases is high due to their isolation and limited access to healthcare resources (42). In such settings, vector control strategies may be the only effective means of preventing disease transmission. For example, in the Pacific Islands, where mosquito-borne diseases such as dengue and Zika are endemic, entomological surveillance has been a key component of successful disease control programs (43). Without such programs, these islands would remain at high risk for disease outbreaks (44).

The results gained from the questionnaires present a nuanced picture of the community's preparedness in addressing the prevalent mosquito issue on Kastellorizo Island. While a substantial proportion of participants reported heightened awareness, with 90.32% actively taking measures to protect against mosquitoes, a concerning majority (70%) expressed dissatisfaction with the perceived effectiveness of these measures. These findings align with the recognition of the need for effective mosquito control strategies, emphasizing the significance of refining public education campaigns and enhancing the efficacy of protective measures (45). The demonstrated gaps in knowledge regarding mosquito breeding sites and biting habits among participants highlight the need for targeted educational initiatives to improve community awareness, echoing the recommendations of Bartlett-Healy et al. (2012) (46). The financial commitment to personal protection, exceeding the minimum wage for a majority (73.91%) of participants, emphasizes the economic burden imposed by the mosquito issue, reinforcing the importance of sustainable mosquito control strategies (47).

These findings underscore the critical importance of engaging the community in entomological surveillance and control efforts. In line with recent research (26,27), fostering a more informed and unified community approach to mosquito control is paramount for fortifying the island's preparedness and

297 resilience against the persistent challenge of mosquitoes. Furthermore, the study's insights into the pivotal
298 role of public awareness emphasizes the integral nature of community support and participation in
299 ensuring the success and sustainability of entomological surveillance and control programs (48). Effective
300 communication strategies, as highlighted by Wong et al. (2014) (49), become instrumental in enhancing
301 public knowledge and participation in vector control efforts, further supporting the need for
302 comprehensive and community-centric approaches to address the mosquito-related challenges on
303 Kastellorizo Island.

304 It's important to highlight that there were no existing baseline data on local species composition
305 and mosquito abundance, primarily due to the remote nature of the current island. Our small-scale study
306 underscores the significance of implementing surveillance and data collection measures as integral
307 components of the control initiative. The results obtained from this small-scale study serve both as
308 preliminary insights and as a foundation for more extensive vector studies that will cover a more extended
309 period and include additional sampling sites. The data obtained from this study can highlight the
310 significance of conducting entomological studies in remote areas and the knowledge gained is valuable
311 contributing to the implementation of mosquito management programs and public health actions. As
312 future actions, we are in the process of designing a tailored approach for a citizen science initiative,
313 utilizing the Mosquito Alert phone application (50). This approach aims to actively involve the community
314 in mosquito monitoring, fostering a collaborative effort in the management and control of mosquito-
315 related issues on the island.

316

Acknowledgments

We would like to thank Stratos Amigdalos and Nikos Asvestis, deputy Mayors for their permission to conduct our experiment in Kastellorizo. We are also very grateful to the agronomists of AgroSpeCom, Dimitris Chouliaras and Giannis Aivazidis for their support of this work.

References

1. WHO. Global vector control response 2017–2030 [Internet]. [cited 2023 Apr 2]. Available from: https://www.who.int/publications-detail-redirect/9789241512978

2. Semenza JC, Menne B. Climate change and infectious diseases in Europe. Lancet Infect Dis. 2009 Jun;9(6):365–75.

3. Fauci AS, Morens DM. Zika Virus in the Americas. Yet Another Arbovirus Threat. N Engl J Med. 2016 Jan;374(7):601–4.

4. Kraemer MUG, Reiner RC, Brady OJ, Messina JP, Gilbert M, Pigott DM, et al. Past and future spread of the arbovirus vectors *Aedes aegypti* and *Aedes albopictus*. Nat Microbiol. 2019 May;4(5):854–63.

5. Messina JP, Brady OJ, Golding N, Kraemer MUG, Wint GRW, Ray SE, et al. The current and future global distribution and population at risk of dengue. Nat Microbiol. 2019 Sep;4(9):1508–15.

6. Sukhralia S, Verma M, Gopirajan S, Dhanaraj PS, Lal R, Mehla N, et al. From dengue to Zika: the wide spread of mosquito-borne arboviruses. Eur. J. Clin. Microbiol. Infect. Dis. 2019;38(1):3–14.

7. Benedict MQ, Levine RS, Hawley WA, Lounibos LP. Spread of the tiger: global risk of invasion by the mosquito *Aedes albopictus*. Vector Borne Zoonotic Dis. 2007;7(1):76–85.

8. Kamgang B, Wilson-Bahun TA, Irving H, Kusimo MO, Lenga A, Wondji CS. Geographical distribution of *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) and genetic diversity of invading population of *Ae. albopictus* in the Republic of the Congo. Wellcome Open Res. 2018 Dec 28;3:79.

9. Medlock J, Gandy S, Johnston C, Handfield Z, Neely K, Vaux A. Snap-shot assessment of adult mosquito (Diptera: Culicidae) densities on the Turks and Caicos Islands, February 2022. JEMCA. 2022 Dec 23;41:1–8.

10. ECDC. *Aedes aegypti* - Factsheet for experts [Internet]. 2023 [cited 2023 Sep 25]. Available from: https://www.ecdc.europa.eu/en/disease-vectors/facts/mosquito-factsheets/aedes-aegypti

11. ECDC. *Aedes albopictus* - Factsheet for experts [Internet]. 2016 [cited 2023 Sep 25]. Available from: https://www.ecdc.europa.eu/en/disease-vectors/facts/mosquito-factsheets/aedes-albopictus

12. Brady OJ, Godfray HCJ, Tatem AJ, Gething PW, Cohen JM, McKenzie FE, et al. Vectorial capacity and vector control: reconsidering sensitivity to parameters for malaria elimination. Trans. R. Soc. Trop. Med. Hyg. 2016 Feb 1;110(2):107–17.

13. Hu W, Clements A, Williams G, Tong S. Dengue fever and El Nino/Southern Oscillation in Queensland, Australia: a time series predictive model. Occup Environ Med. 2010 Dec;67(5):307–11.

14. Barrera R, Amador M, Clark GG. Use of the pupal survey technique for measuring *Aedes aegypti* (Diptera: Culicidae) productivity in Puerto Rico. Am J Trop Med Hyg. 2006 Feb;74(2):290–302.

15. Farajollahi A, Kesavaraju B, Price DC, Williams GM, Healy SP, Gaugler R, et al. Field efficacy of BG-Sentinel and industry-standard traps for *Aedes albopictus* (Diptera: Culicidae) and West Nile virus surveillance. J Med Entomol. 2009 Jul;46(4):919–25.

16. Reiskind MH, Lounibos LP. Effects of intraspecific larval competition on adult longevity in the mosquitoes *Aedes aegypti* and *Aedes albopictus*. Med Vet Entomol. 2009 Mar;23(1):62–8.

17. Dowling Z, Armbruster P, LaDeau SL, DeCotiis M, Mottley J, Leisnham PT. Linking mosquito infestation to resident socioeconomic status, knowledge, and source reduction practices in suburban Washington, DC. Ecohealth. 2013 Mar;10(1):36–47.

18. Morse W, Izenour K, McKenzie B, Lessard S, Zohdy S. Perceptions and practices of mosquito-borne diseases in Alabama – is concern where it should be? BMC Public Health. 2019 Jul 23;19(1):987.

19. Stefopoulou A, Balatsos G, Petraki A, LaDeau SL, Papachristos D, Michaelakis A. Reducing *Aedes albopictus* breeding sites through education: A study in urban area. PLOS ONE. 2018 Oct 8;13(11):e0202451.

20. Giatropoulos A, Michaelakis A, Pontikakos C. Records of *Aedes albopictus* and *Aedes cretinus* (Diptera: Culicidae) in Greece from 2009 to 2011. HPPJ 2012 Jul 1;5:49–56.

21. Medlock JM, Hansford KM, Versteirt V, Cull B, Kampen H, Fontenille D, et al. An entomological review of invasive mosquitoes in Europe. Bull Entomol Res. 2015 Dec;105(6):637–63.

22. Badieritakis E, Papachristos D, Latinopoulos D, Stefopoulou A, Kolimenakis A, Bithas K, et al. *Aedes albopictus* (Skuse, 1895) (Diptera: Culicidae) in Greece: 13 years of living with the Asian tiger mosquito. Parasitol Res. 2018 Feb;117(2):453–60.

23. Akiner MM, Demirci B, Babuadze G, Robert V, Schaffner F. Spread of the Invasive Mosquitoes *Aedes aegypti* and *Aedes albopictus* in the Black Sea Region Increases Risk of Chikungunya, Dengue, and Zika Outbreaks in Europe. PLOS Negl Trop Dis. 2016 Apr;10(4):e0004664–e0004664.

24. Fotakis EA, Mavridis K, Kampouraki A, Balaska S, Tanti F, Vlachos G, et al. Mosquito population structure, pathogen surveillance and insecticide resistance monitoring in urban regions of Crete, Greece. PLOS Negl Trop Dis. 2022 Feb 17;16(2):e0010186.

25. ELSTAT. Hellenic Statistical Authority. 2018. [cited 2023 Sep 25]. Available from: www.elstat.gr

26. Kolimenakis A, Tsesmelis D, Richardson C, Balatsos G, Milonas PG, Stefopoulou A, et al. Knowledge, Attitudes and Perception of Mosquito Control in Different Citizenship Regimes within and Surrounding the Malakasa Open Accommodation Refugee Camp in Athens, Greece. Int J Environ Res Public Health. 2022 Dec 16;19(24):16900.

27. Stefopoulou A, LaDeau SL, Syrigou N, Balatsos G, Karras V, Lytra I, et al. Knowledge, Attitude, and Practices Survey in Greece before the Implementation of Sterile Insect Technique against *Aedes albopictus*. Insects. 2021 Mar 2;12(3):212.

28. Beleri S, Balatsos G, Karras V, Tegos N, Sereti F, Rachiotis G, et al. Seasonal Phenological Patterns and Flavivirus Vectorial Capacity of Medically Important Mosquito Species in a Wetland and an Urban Area of Attica, Greece. TropicalMed. 2021 Sep 28;6(4):176.

29. Bisia M, Papadopoulos P, Filis S, Beleri S, Tegos N, Lamprou GK, et al. Field Evaluation of Commonly Used Adult Mosquito Traps in Greece. Vector Borne Zoonotic Dis. 2023 Mar;23(3):119–28.

30. EU Health Gateways [internet]. Recommendations for standar operating procedures (SOPs) development for vector (mosquito) surveillance and control activities at ports and airports 2022. [cited 2023 Sep 25]. Available from: https://www.healthygateways.eu/Portals/0/plcdocs/D9_2_EU_HG_SOPs.pdf?ver=2023-02-01-181716-890

31. WHO [internet]. Entomological surveillance for *Aedes* spp. in the context of Zika virus. 2016; [cited 2023 Sep 25]. Available from: https://www.who.int/publications/i/item/WHO-ZIKV-VC-16.2

32. Bellini R, Michaelakis A, Petrić D, Schaffner F, Alten B, Angelini P, et al. Practical management plan for invasive mosquito species in Europe: I. Asian tiger mosquito (*Aedes albopictus*). Travel Med Infect Dis. 2020;35:101691.

33. Becker N, Petric D, Zgomba M, Boase C, Madon M, Dahl C, et al. Mosquitoes and their control. Springer Science & Business Media; 2010.

34. Samanidou-Voyadjoglou A, Harbach RE. Keys to the adult female mosquitoes (Culicidae) of Greece. Eur. Mosq. Bull. 2001;10:13–20.

35. Patsoula E, Samanidou-Voyadjoglou A, Spanakos G, Kremastinou J, Nasioulas G, Vakalis NC. Molecular characterization of the *Anopheles maculipennis* complex during surveillance for the 2004 Olympic Games in Athens. Med Vet Entomol. 2007 Mar;21(1):36–43.

36. Patsoula E, Samanidou-Voyadjoglou A, Spanakos G, Kremastinou J, Nasioulas G, Vakalis NC. Molecular and morphological characterization of *Aedes albopictus* in northwestern Greece and differentiation from *Aedes cretinus* and *Aedes aegypti*. J Med Entomol. 2006 Jan;43(1):40–54.

37. Darsie RFJ, Samanidou-Voyadjoglou A. Keys for the identification of the mosquitoes of Greece. J Am Mosq Control Assoc. 1997 Sep;13(3):247–54.

38. Samanidou-Voyadjoglou A, Patsoula E, Spanakos G, Vakalis N. Confirmation of Aedes albopictus (Skuse) (Diptera: Culicidae) in Greece. Eur. Mosq. Bull. 2005;19:10–1.

39. Vasquez MI, Notarides G, Meletiou S, Patsoula E, Kavran M, Michaelakis A, et al. Two invasions at once: update on the introduction of the invasive species *Aedes aegypti* and *Aedes albopictus* in Cyprus – a call for action in Europe. Parasite. 2023;30:41.

40. Kraemer MUG, Sinka ME, Duda KA, Mylne AQN, Shearer FM, Barker CM, et al. The global distribution of the arbovirus vectors *Aedes aegypti* and *Ae. albopictus*. Elife. 2015 Jun 30;4:e08347.

41. Rezza G, Nicoletti L, Angelini R, Romi R, Finarelli AC, Panning M, et al. Infection with chikungunya virus in Italy: an outbreak in a temperate region. Lancet. 2007 Dec 1;370(9602):1840–6.

42. Wilder-Smith A, Quam M, Sessions O, Rocklov J, Liu-Helmersson J, Franco L, et al. The 2012 dengue outbreak in Madeira: exploring the origins. Euro Surveill. 2014 Feb 27;19(8):20718.

43. Vontas J, Kioulos E, Pavlidi N, Morou E, della Torre A, Ranson H. Insecticide resistance in the major dengue vectors *Aedes albopictus* and *Aedes aegypti*. Pestic Biochem Phys. 2012 Oct 1;104(2):126–31.

44. Beebe NW, Ambrose L, Hill LA, Davis JB, Hapgood G, Cooper RD, et al. Tracing the tiger: population genetics provides valuable insights into the *Aedes* (Stegomyia) *albopictus* invasion of the Australasian Region. PLOS Negl Trop Dis. 2013 Jan 1;7(8):e2361.

45. Stoddard ST, Morrison AC, Vazquez-Prokopec GM, Soldan VP, Kochel TJ, Kitron U, et al. The Role of Human Movement in the Transmission of Vector-Borne Pathogens. PLOS Negl Trop Dis. 2009 Jun 21;3(7):e481.

46. Bartlett-Healy K, Unlu I, Obenauer P, Hughes T, Healy S, Crepeau T, et al. Larval mosquito habitat utilization and community dynamics of *Aedes albopictus* and *Aedes japonicus* (Diptera: Culicidae). J Med Entomol. 2012 Jul;49(4):813–24.

47. Roiz D, Wilson AL, Scott TW, Fonseca DM, Jourdain F, Müller P, et al. Integrated *Aedes* management for the control of *Aedes*-borne diseases. PLOS Negl Trop Dis. 2018 Nov 6;12(12):e0006845.

48. Andersson N, Nava-Aguilera E, Arosteguí J, Morales-Perez A, Suazo-Laguna H, Legorreta-Soberanis J, et al. Evidence based community mobilization for dengue prevention in Nicaragua and Mexico (Camino Verde, the Green Way): cluster randomized controlled trial. BMJ. 2015 Jul 8;351:h3267.

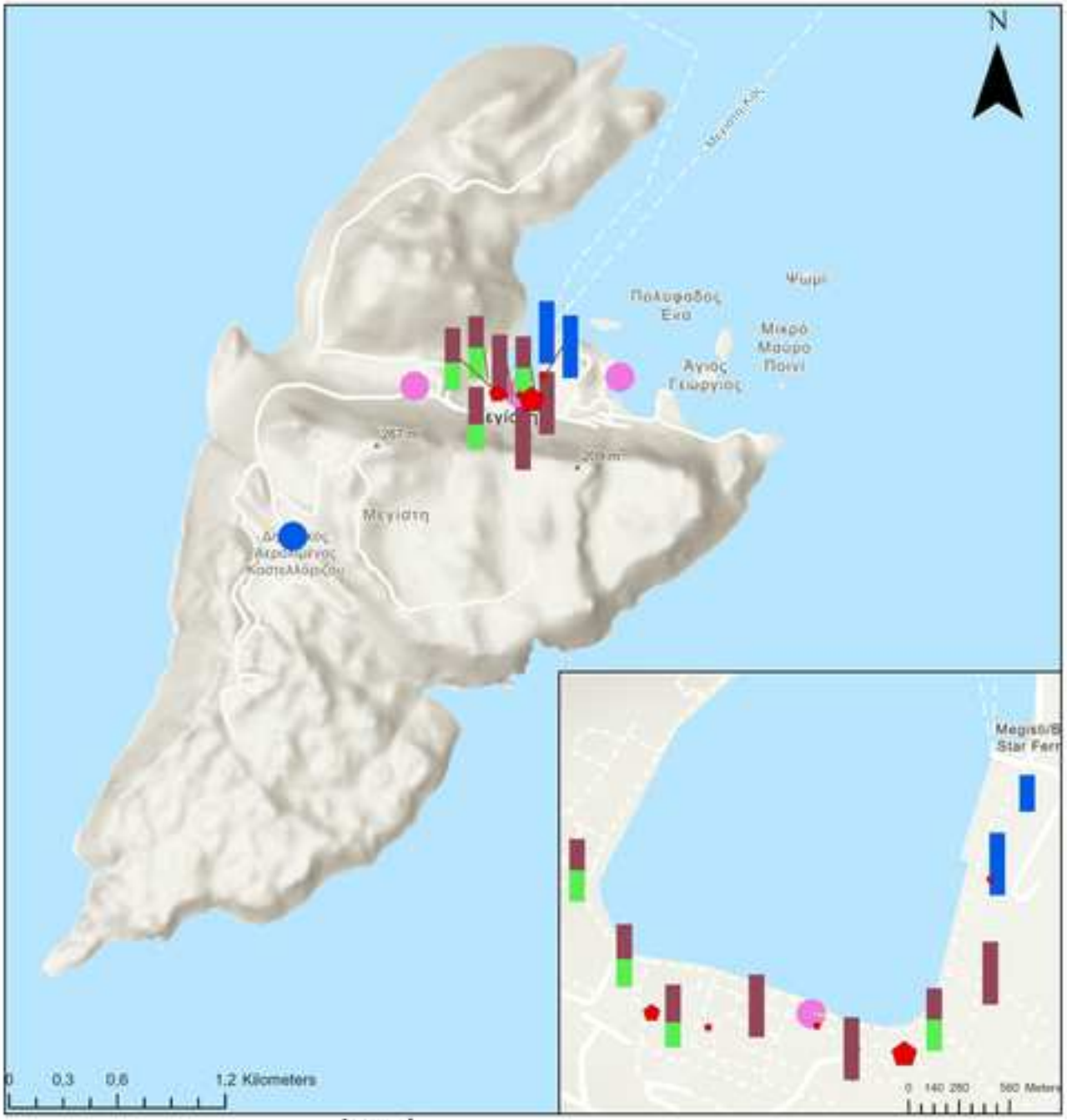
49. Wong LP, AbuBakar S, Chinna K. Community Knowledge, Health Beliefs, Practices and Experiences Related to Dengue Fever and Its Association with IgG Seropositivity. PLOS Negl Trop Diss. 2014;8(5):e2789.

50. Južnič-Zonta Ž, Sanpera-Calbet I, Eritja R, Palmer JRB, Escobar A, Garriga J, et al. Mosquito alert: leveraging citizen science to create a GBIF mosquito occurrence dataset. Gigabyte. 2022 May 30;2022:1–11.

Figure 1

Click here to access/download;Figure;Figure 1.tif

Entomological surveillance in the island of Megisti: collection of *Ae. albopictus* eggs, male/ female *Ae. albopictus* with HLC and collection of male/female *Cx. pipiens* & *Ae. albopictus* with BG- sentinel traps (equipped with BG lure)



Legend

OVI traps

No. of eggs

- 2 - 3
- 4 - 32
- 33 - 164

Human Landing Collection

- Female Ae. albopictus
- Male Ae. albopictus
- Absense of Ae. albopictus

BG traps

- Cx. pipens
- Ae. albopictus
- Absense

Figure 2

Click here to access/download;Figure;Figure 2.tif

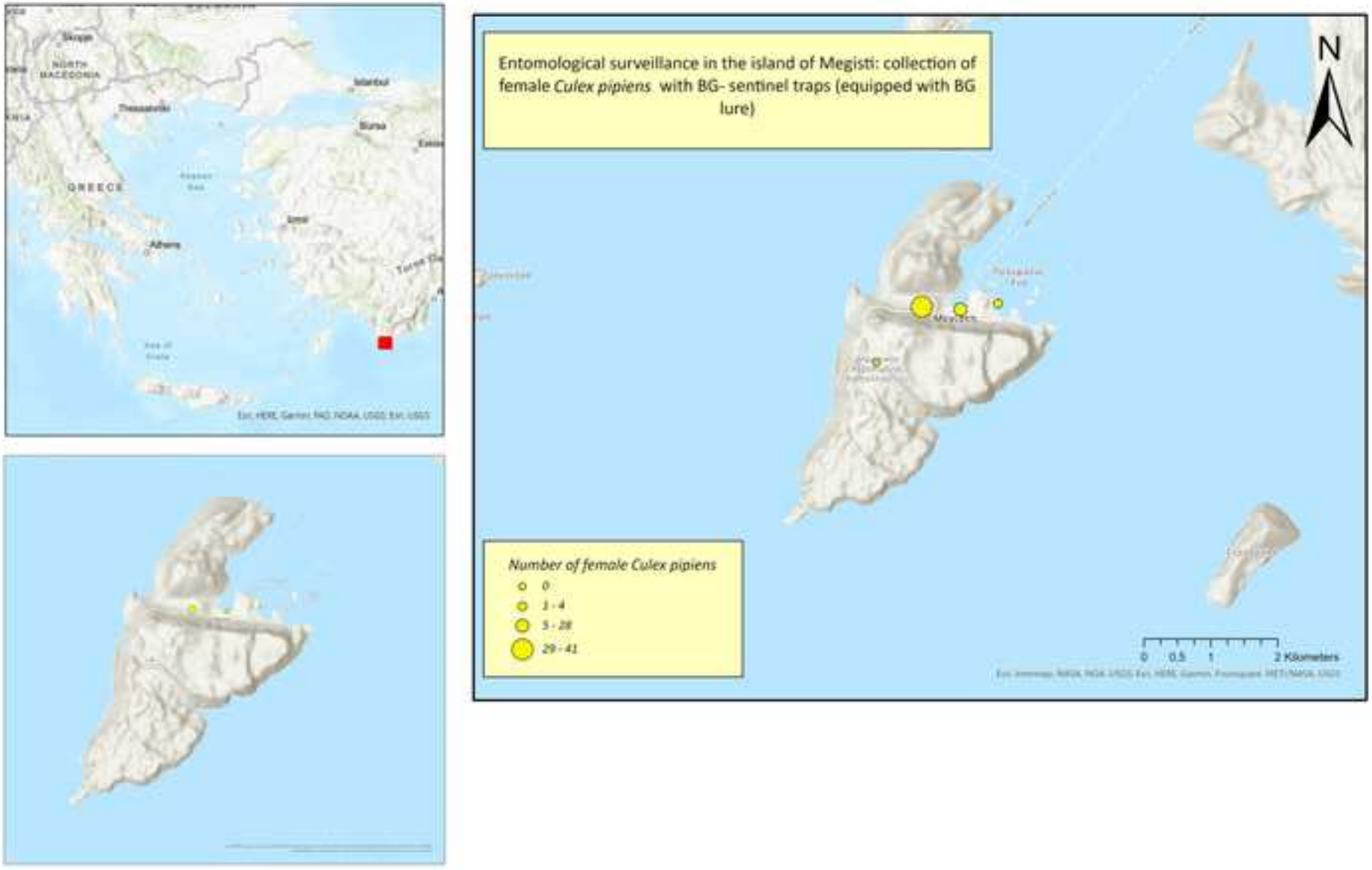


Figure 3

Click here to access/download;Figure;Figure 3.tif