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JOURNAL OF THE EUROPEAN MOSQUITO CONTROL ASSOCIATION
(2024) 1–15

BRILL
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ACADEMIC

RESEARCH ARTICLE

A survey targeting exotic *Aedes* mosquito species in Central Europe, summer 2023, reveals the extensive occurrence of *Aedes japonicus* in Poland

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Received 22 April 2024 | Accepted 17 June 2024 | Published online 22 August 2024

Abstract

In the frame of the entomological VectorNet network and its capacity building activities, we collected original mosquito distribution data in southern Poland and bordering areas of the Czech Republic, Germany and Slovakia, in June and September–November 2023. Because of the suspected occurrence of *Aedes japonicus* or *Ae. koreicus* in Poland, provided by a photo posted early 2022 on iNaturalist, we targeted the exotic *Aedes* species in our sampling strategy, but also collected data on other mosquito species. Besides some adult catches, we mainly collected mosquito immature stages from artificial and natural water containers but occasionally from other aquatic habitats. In addition, we collated citizen data and modelled the distribution of *Ae. japonicus* in Europe incorporating the newly collected data. During this snapshot field study, a total of 162 samples, including 139 yielding mosquitoes, were taken from 111 locations across 47 administrative units, resulting on the detection of 22 mosquito taxa. Our study provides the first substantiated records of *Ae. japonicus* and *Anopheles petragnani* in Poland (the second confirmed by molecular identification). While *Ae. japonicus* is clearly established over a large part of the country, no other exotic mosquito species was detected. The presence of *Ae. japonicus* was also confirmed at one location by four citizen records submitted to MosquitoAlert in 2023. Regarding native mosquitoes, we identified their presence in 127 species/NUTS3 combinations (113 for Poland, including a single record for *An. petragnani*). An updated modelling of the distribution of *Ae. japonicus* suggests higher environment suitability in Central and Eastern Europe than has been previously estimated. *Aedes japonicus* is probably widespread in the Czech Republic and Slovakia, and might soon colonise the bordering region of Ukraine. Its establishment extends the putative mosquito vector list for West Nile and Rift Valley fever viruses in Central Europe.

Published with license by Koninklijke Brill BV | DOI:10.52004/2054930X-20241010
 | ISSN: 2054-930X (online)

Keywords

Culicidae – first report – *Anopheles petragnani* – Czech Republic – Germany

1 Introduction

Mosquito distribution data are useful to assess the public and veterinary health risk related to pest or vector species, and they are relevant to understanding mosquito diversity and biology in a local context. This is of particular importance for invasive species of which the distribution range is rapidly changing, introducing pathogen-transmission risk in naïve areas (Schaffner *et al.*, 2013). Mosquito species occurrence data must therefore be collected by field scientists and shared to produce updated distribution maps. This is a major aim for the VectorNet Project (https://www.ecdc.europa.eu/en/about-us/partnerships-and-networks/disease-and-laboratory-networks/vector-net), an entomological network funded by the European Centre for Disease Prevention and Control (ECDC) and the European Food Safety Authority (EFSA). This network produces pan-European vector distribution maps to facilitate preparedness and response for vector-borne diseases (Braks *et al.*, 2022). The present study, commissioned by VectorNet, had the double objective of capacity building and field data collection, encouraging mosquito surveillance and filling gaps in vector distribution maps.

In 2020, a VectorNet survey was carried out to collect data about existing vector surveillance activities in Europe and neighbouring countries. The outcomes showed that very little mosquito surveillance was completed in Poland both for exotic *Aedes* mosquito species (https://www.ecdc.europa.eu/en/publications-data/invasive-mosquito-surveillance-effort-2015-2019) and for native mosquito species (https://www.ecdc.europa.eu/en/publications-data/seasonal-active-surveillance-native-mosquitoes-2017-2019). The mosquito fauna of Poland is currently known to comprise 47 species, but no exotic *Aedes* have yet been detected (Robert *et al.*, 2019). Recent findings mean that *Anopheles* (*Anopheles*) *daciae* Linton, Nicolescu & Harbach, 2004 and *An.* (*Ano.*) *hyrcanus* (Pallas 1771) have to be added (Lühken *et al.*, 2023; Rydzanicz *et al.*, 2017). While historical mosquito distributions have been described (Kubica-Biernat, 1999), recent distribution data remain scarce, as illustrated on VectorNet maps (https://www.ecdc.europa.eu/en/disease-vectors/surveillance-and-disease-data/mosquito-maps).

Exotic *Aedes* mosquito (EAM) species are known to have dispersed throughout Europe but no substantiated information on their status in Poland was available at the end of 2022 (ECDC, 2022; Medlock *et al.*, 2012). However, a photo posted on iNaturalist website in January 2022 strongly suggested the occurrence of either *Aedes* (*Hulecoeteomyia*) *japonicus* (Theobald, 1901) or *Ae.* (*Hul.*) *koreicus* (Edwards, 1917) at Bytom, in the south of the country (https://www.inaturalist.org/observations/104207584; Supplementary Figure S1). Therefore, and since little mosquito surveillance is performed and little recent mosquito distribution data exist for Poland, we investigated the possible occurrence of EAMs at suitable sites in southern Poland and in neighbouring areas of Central Europe. We also collected field data for other VectorNet priority species (Wint *et al.*, 2023) in the same areas, in order to update mosquito distribution data sets. We here report the outcomes of the snapshot field study performed.

2 Materials and methods

The VectorNet mosquito capacity building activities in 2023 aimed (1) to establish collaboration and to train Polish scientists in collecting and identifying mosquito vector species, and (2) to investigate the possible occurrence of exotic *Aedes* species in southern Poland. Scientists from three Polish institutes attended the training. The capacity building and data acquisition activities included:

- a theoretical and practical training course on surveillance and identification of mosquito vector species (adult female and larval stages) in Poland, completed at the University of Łódź, 26–29 September 2023;
- two field trips to investigate the occurrence of exotic *Aedes* species in south Poland, from 18 June to 25 June 2023 and from 25 September to 6 October 2023 (the latter as part of the training course);
- additional field sampling by the trained teams at two single locations in the neighbourhoods of Poznań and Puławy, between 3 October and 18 November 2023.

To detect the occurrence of mosquito species, we used various sampling methods whilst travelling across the study area. Because of the travelling, repeated sampling of fixed locations was difficult and so larval sampling was the routine method applied, while complementary investigation was performed by adult catches (including trapping performed mainly for demonstration during the capacity building course and subsequently at one location). The study area was defined to be southern Poland and its neighbouring regions of Czech Republic, Germany and Slovakia. Bytom, the location with the suspected presence of *Ae. japonicus*, was at the top of the list of locations to be visited, and other locations were defined in the neighbouring administrative units. Once *Ae. japonicus* was found in a unit, we moved to the next unit, as long as the field trip duration allowed it. When no *Ae. japonicus* was found, we searched for another suitable location. A location was considered negative when at least 10 containers providing suitable larval habitats were checked and no larvae were found. If three or more locations remained negative, depending to the availability of suitable environment, we moved to the next chosen administrative unit. The approximate mosquito sample sites were identified in advance using Google Earth™ images and finalised on-site by an intuitive and visual selection of sites. Cemeteries, community gardens, industrial and suburban zones, were prioritised as places usually providing many artificial water containers. In addition, some other sites were selected for other VectorNet priority mosquito species, in particular wetlands, using a similar selection process. The two additional sites surveyed by the local teams following the training course were selected because of their proximity to the participant's institute or home.

Mosquitoes were sampled according to standard procedures (Medlock *et al.*, 2018). Mosquito larvae and pupae were collected with an aquatic net and a white plastic tray, and specimens were removed from the water with a pipette and placed in 70% ethanol in a plastic vial. Pupae and in some cases a few 4th instar larvae were kept in water from the source breeding site until the adults emerged. Adult catches were performed by means of CO_2-baited encephalitis vector survey (EVS) traps (BioQuip Products Inc., Rancho Dominiquez, CA, USA), black-hole traps (a trap that combines black-light, heat and a titanium dioxide coated panel that produces CO_2; Terminator trap, Archer, Australia), and BG-Lure® (chemical)-baited BG-Sentinel® traps (Biogents, Regensburg, Germany). For the EVS trap, carbon dioxide was provided either by dry-ice or by an out-of-service fire extinguisher gas bottle equipped with a BG-Timer® and CO_2 release set (Biogents). Larvae and adults were morphologically identified using available identification keys (Becker *et al.*, 2020; Schaffner *et al.*, 2001). Data collection was processed using the VECMAP® software package (AVIA-GIS, Zoersel, Belgium).

Morphological identification was supplemented by genetic analysis to determine the species identity of members of the Maculipennis and the Claviger complexes of the genus *Anopheles*. DNA was isolated from 2 legs or an abdomen mechanically removed from a mosquito in a TissueLyser II (Qiagen, Hilden, Germany) and following the manual instructions of the Sherlock AX kit (A&A Biotechnology, Gdańsk, Poland). DNA extracts were quantified using a Drop-Sense 16 spectrophotometer (Trinean, Gentbrugge, Belgium). DNA amplification of the ribosomal internal transcribed spacer 2 (ITS2) was performed as described by Linton *et al.* (2001), using the 5.8SF and 28SR primers recommended by Collins and Paskewitz (1996). PCR products were purified following the protocol of the Agencourt AMPure XP PCR Purification kit (Beckman Coulter, Brea, CA, USA). The Promega ProDye™ Terminator sequencing system was applied in-house for DNA sequencing. Each PCR contained 4 µl purified DNA, 0.5 µl ProDye Master Mix, 2 µl sequencing buffer, 0.25 µl sequencing primer (10 µM) and 3.25 µl nuclease free water. PCRs started with 1 min initial denaturation at 96 °C, followed by 25 cycles of denaturation at 96 °C for 10 s, annealing at 50 °C for 5 s and extension at 60 °C for 4 min. PCRs were run in a Mastercycler Nexus (Eppendorf, Hamburg, Germany). For sequencing, PCR products were purified following the manual instructions of the Agencourt CleanSEQ Dye-Terminator Removal protocol (Beckman Coulter). PCR products were sequenced using an ABI 3730XL capillary sequencer (Applied Biosystems, Foster City, CA, USA). All laboratory steps were conducted in the facilities of the National Museum of Natural History, Luxembourg. Sequences were quality checked, edited and trimmed in Geneious R6 version 6.1.8 (Kearse *et al.*, 2012) and compared with sequences in the GenBank database by the BLAST® online tool. To discriminate between *Anopheles daciae* and *An.* (*Ano.*) *messeae* Falleroni, 1926, aligned sequences were visually checked for the presence of the five species-specific diagnostic polymorphic sites (Brusentsov *et al.*, 2023; Nicolescu *et al.*, 2004).

The data set was completed by citizen science data reported through the Mosquito Alert platform (https://www.mosquitoalert.com; Palmer *et al.*, 2017). This platform was created in 2014 and is based on a cell phone tool used to report invasive species by picture(s), location and (anonymous) user comment. These reports are received in a dedicated server where they are in the first place automatically assessed by an Artificial Intelligence (AI) unit which has the capacity to emit preliminary alerts. Later, reports are analysed by a human team of ca. 109 experts across the world. Once a report is validated by three experts the system averages their outputs and the report is published on a map in https://map.mosquitoalert.com/, from where all data can be freely downloaded. Sampling effort by citizens on a given area is estimated as the apps send geolocations several times across the day.

The updated distribution data were subjected to spatial distribution modelling using Boosted regression Tree and Random Forest techniques – both well-established standard modelling methods – and a standardised suite of environmental, ecological, demographic, and remotely sensed climatic and vegetation parameters subjected to temporal Fourier analysis. The outputs from each method were averaged to provide a single ensembled output. The methods and covariates are adapted from those set out in detail in Scharlemann *et al.* (2008), Wint *et al.* (2022), and Messina and Wint (2024).

3 Results

Field sampling effort

A total of 162 samples, including 139 (85.8%) yielding mosquitoes, were taken from 111 locations across 47 administrative units (statistical units NUTS3; Supplementary Table S3; Figure 1). Most of the locations were from Poland (86.5%) but a few from neighbouring parts of Germany (8.1%), the Czech Republic (4.5%), and Slovakia (0.9%; Table 1).

Field sampling data

Except in Puławski where a trap was run over seven weeks at the same location, yielding a total of 27 catches, from one to five samples were taken in each investigated administrative unit. The large majority of these samples were of aquatic nature, containing immature mosquito stages (n=114; 70.4%), while only a few samples were obtained by trapping adults (n=33; 20.4%) and manual adult catches (n=15; 9.2%) (Table 1). The latter comprised human landing (n=13) and resting (n=2) catches, and the former were based on black light/CO_2-baited (n=27), CO_2-baited (n=4) and lure-baited (n=2) traps run at the training location and at a local team's location near Puławy. Of all aquatic larval habitats sampled in our study, most were suitable for container-inhabiting species (n=97; 85.1%), with artificial and natural containers representing 78.9% and 6.1%, respectively (Figure 2). All samples with no mosquitoes (n=19) belonged to the category 'human-made containers' and were located mainly in Poland (n=16) but also in the Czech Republic (n=2) and Germany (n=1).

Overall, we collected mosquitoes from at least one location per unit in a total of 47 NUTS3 administrative units from four countries (Table 2). In Poland, we detected at least one mosquito species in 13 of 17 NUTS2 units (provinces; 76.5%), and in 39 of 73 NUTS3 units (53.4%). Details of findings per NUTS3 unit and per species are provided in Tables S3–S4. In total, we observed and identified 80 mosquito eggs, 2,192 larvae, 213 pupae, 167 males and 582 females. Among the adults, 109 males and 133 females were obtained from reared larvae or pupae. Details of all field observations are given in Supplementary Table S6.

A total of 22 mosquito species/taxa were caught in the 139 positive samples. The most frequently encountered

TABLE 1 Sampling effort of our snapshot mosquito field study in Central Europe, 2023.[1]

Country	No. of admin units (NUTS3)	No. of locations	No. of samples	No. of neg. immature samples	No. of pos. immature samples	No. of adult catches[2]	No. of neg. adult traps set	No. of pos. adult traps set
Czech Republic	3	5	5	2	3	0	0	0
Germany	4	9	10	1	9	0	0	0
Poland	39	96	146	16	82	15	4	29
Slovakia	1	1	1	0	1	0	0	0
Total	47	111	162	19	95	15	4	29

[1] Pos.= mosquitoes detected; neg. = no mosquito detected.
[2] All positive.

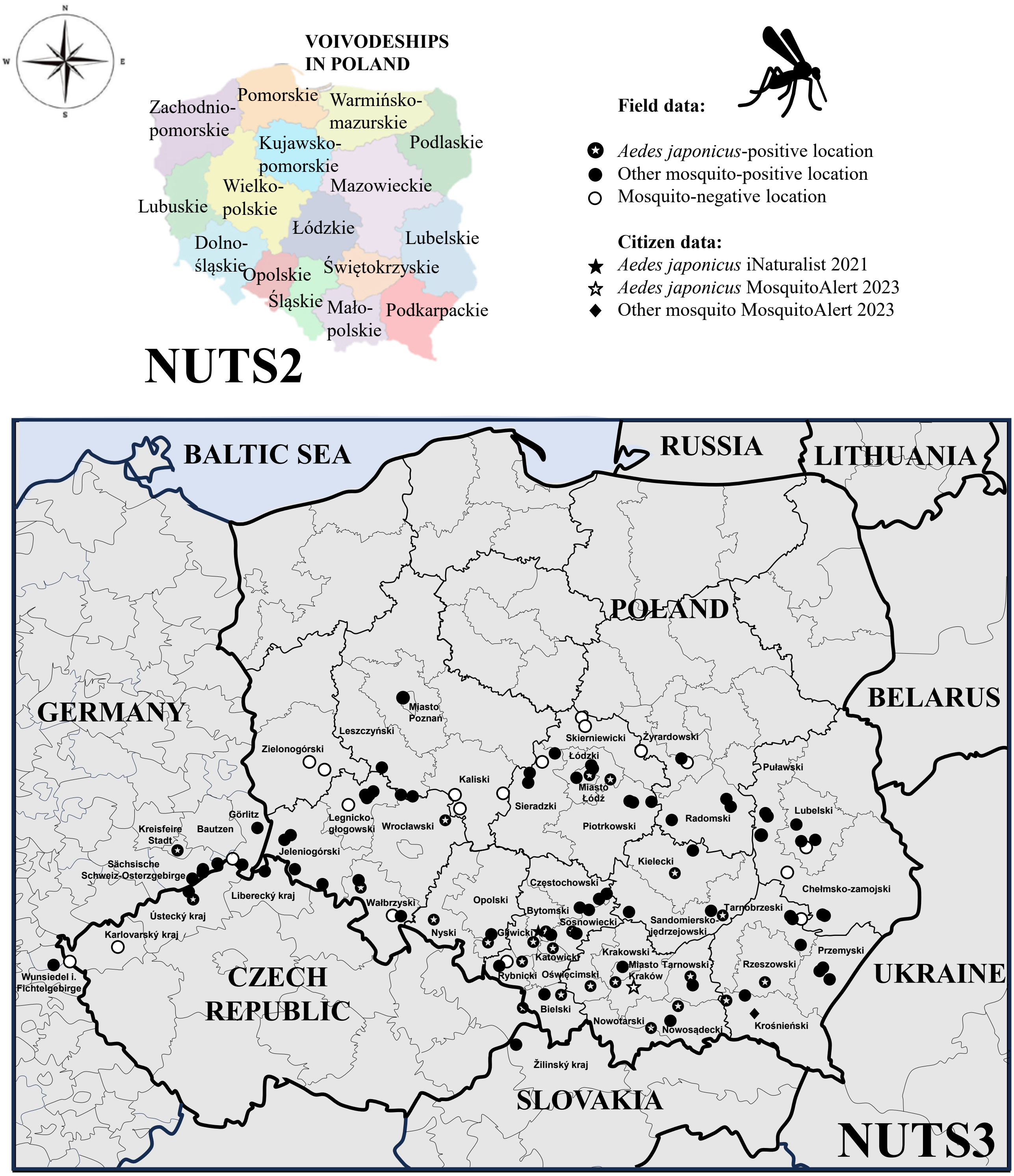

FIGURE 1 Location of sampling sites of our snapshot mosquito field study in Central Europe, 2023, with occurrences of *Aedes japonicus* and other mosquito species.

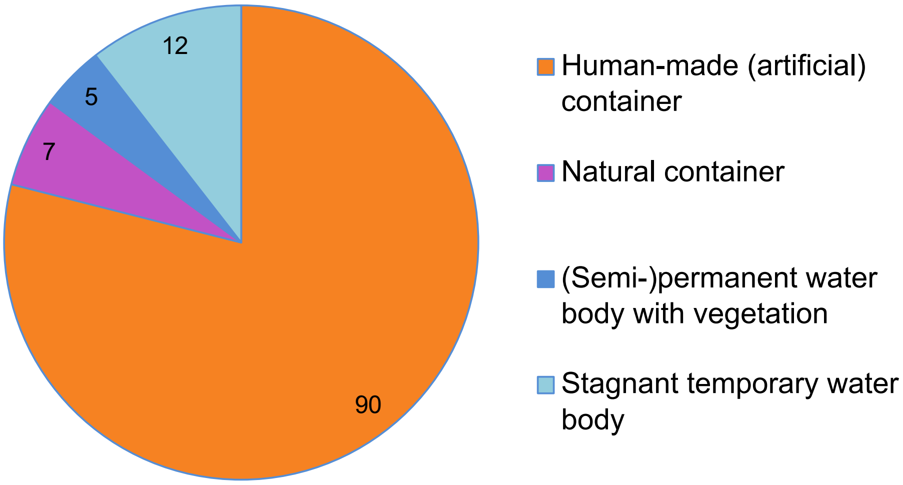

FIGURE 2 Types of aquatic larval habitats sampled in our snapshot mosquito field study in Central Europe, 2023.

TABLE 2 Number of administrative units positive for a mosquito species at one or more locations per country and admin/statistical units in our snapshot mosquito field study in Central Europe, 2023 (total number of positive locations = 90).

Country	Czech Republic		Germany		Poland		Slovakia		Overall	
Admin/statistical units (NUTS)	N2	N3	N2	N3	N2	N3	N2	N3	N2	N3
Aedes (*Aedes*) *cinereus/geminus*					3	4			3	4
Aedes (*Aed.*) *geminus* Peus, 1970					1	1			1	1
Aedes (*Aedimorphus*) *vexans* (Meigen, 1830)					6	6			6	6
Aedes (*Dahliana*) *geniculatus* (Olivier, 1791)	1	1	1	2	5	7			7	10
Aedes (*Hulecoeteomyia*) *japonicus* (Theobald, 1901)	2	2	1	1	8	21			11	24
Aedes (*Ochlerotatus*) *annulipes/cantans*					3	5			3	5
Aedes (*Och.*) *sticticus* (Meigen, 1838)					3	4			3	4
Anopheles (*Anopheles*) *claviger s.s.* (Meigen, 1804)					5	10			5	10
Anopheles (*Ano.*) *daciae* Linton, Nicolescu & Harbach, 2004					1	1			1	1
Anopheles (*Ano.*) *maculipennis s.l.* Meigen, 1818					1	1			1	1
Anopheles (*Ano.*) *maculipennis s.s.* Meigen, 1818					4	4			4	4
Anopheles (*Ano.*) *messeae* Falleroni, 1926					1	1			1	1
Anopheles (*Ano.*) *petragnani* Del Vecchio, 1939					1	1			1	1
Anopheles (*Ano.*) *plumbeus* Stephens, 1828			1	2	8	13			9	15
Coquillettidia (*Coquillettidia*) *richiardii* (Ficalbi, 1889)					1	1			1	1
Culex (*Culex*) *pipiens* L., 1758			1	1	9	16			10	17
Culex (*Cux.*) *pipiens/torrentium*			1	3	12	20	1	1	14	24
Culex (*Cux.*) *torrentium* Martini, 1925			2	3	2	2			4	5
Culex (*Maillotia*) *hortensis* Ficalbi, 1889					1	1			1	1
Culex (*Neoculex*) *territans* Walker, 1856					4	4			4	4
Culiseta (*Culiseta*) *annulata* (Schrank, 1776)			1	1	9	10			10	11
Culiseta (*Cus.*) *glaphyroptera* (Schiner, 1864)					1	1			1	1
No. of units surveyed	2	3	2	4	13	39	1	1	18	47

taxon was *Cx. pipiens/torrentium* (as unsorted sister species) with 42 occurrences, plus 24 occurrences of *Cx. pipiens* and 5 of *Cx. torrentium*, in samples providing species-identifiable adults. These two species were found developing together at one location only. The following more frequent species were *Ae. japonicus* (24 locations), followed by *An. plumbeus* (18 locations) and *Ae. geniculatus* (13 locations). All these taxa were frequently collected from aquatic container habitats (Figure 3). The remaining less frequently encountered species were either rarely found in such containers (i.e. *An. claviger sensu stricto* (*s.s.*), *An. petragnani*, *Cs. annulata*, *Cs. glaphyroptera*, *Cx. hortensis, Cx. territans*) or collected from stagnant temporary or (semi-)permanent water bodies (i.e. *Ae. cinereus/geminus*, *Ae. geminus*, *Ae. sticticus*, *Ae. vexans, An. maculipennis sensu lato* (*s.l.*)) or as biting adults only (i.e. *Ae. annulipes/cantans*, *Cq. richiardii*).

Molecular identification of specimens

Immatures belonging to the Claviger Complex were found at 12 locations and in one case, morphological characters suggested the identification of *An. petragnani*. To strengthen the identification, we conducted ITS2 sequencing on a specimen from that sample as well as from two other samples showing typical *An. claviger* morphology. The sequences obtained from the latter two specimens showed 100% similarities with several sequences of *An. claviger s.s.*, while the one obtained from the suspected *An. petragnani* showed 97.4% similarities with the single sequence of that species deposited in GenBank and with sequences from other studies (FS, unpublished), but 86.5% only with sequences of *An. claviger s.s.*. However, that sequence showed almost 100% similarities with two other sequences identified as *An. petragnani*, obtained from a specimen collected in Luxembourg (BOLD ID: MNHNL172-22; Schaffner *et al.*,

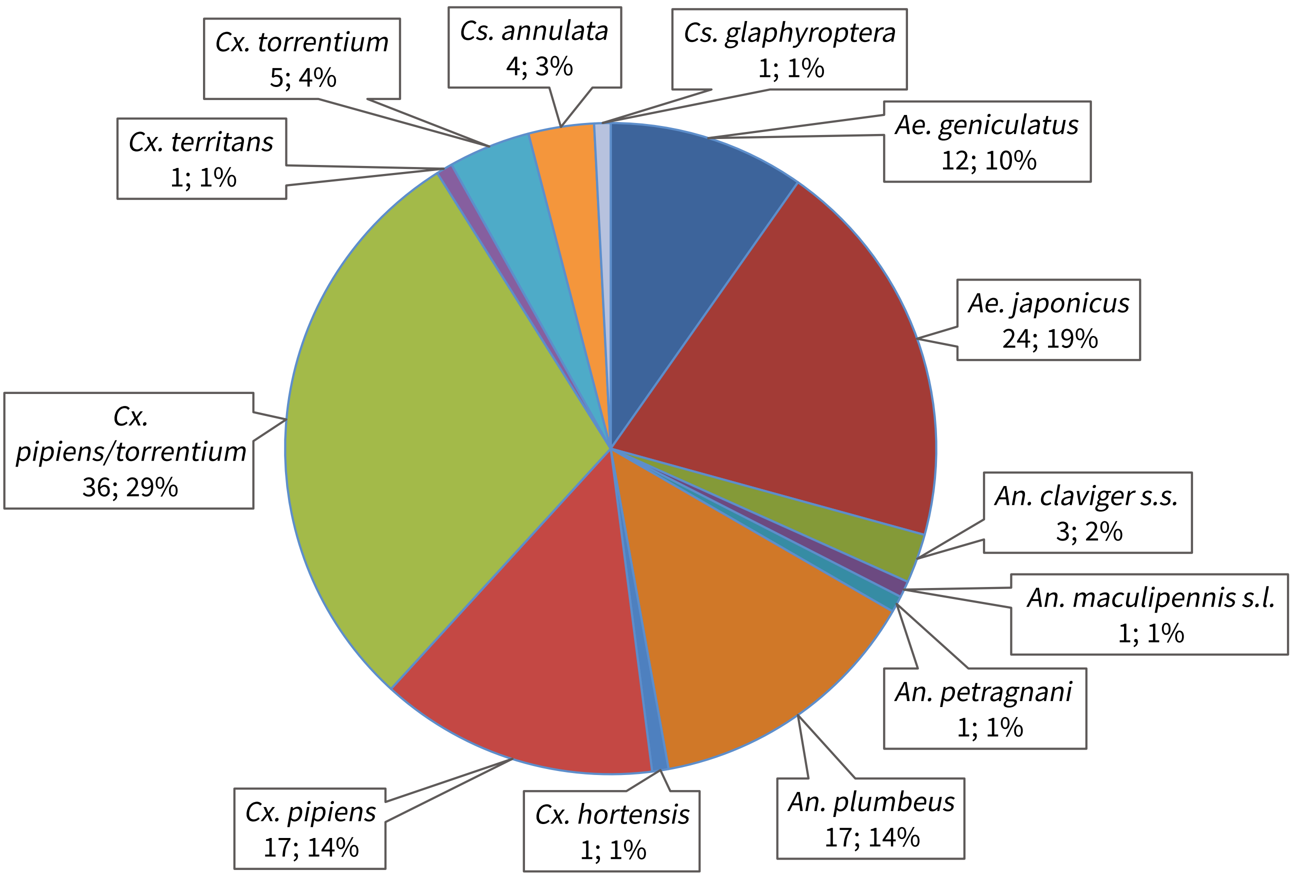

FIGURE 3 Frequency of mosquito species found in aquatic artificial and natural containers in our snapshot mosquito field study in Central Europe, 2023. Total number of mosquito-positive container samples = 78.

2023) and from several specimens collected in Germany (consensus sequence; Becker *et al.*, 2016). These sequences show the similar nucleotide differences with other sequences of *An. claviger s.s.*.

Larvae belonging to the Maculipennis Complex were found at five locations in six samples of 2 to 4 larvae each, and ITS2 sequencing was conducted on 1 specimen from each of these samples. Among the sequences obtained, four showed 100% similarities with sequences of *An. maculipennis s.s.*. One of the two remaining sequences showed 100% similarities with various sequences of both *An. daciae* and *An. messeae*, while the second showed 99.5% and 100% similarities, respectively. The visual check of the sequences aligned with sequences of both species from various countries allowed us to confirm the second sequence to belong to *An. messeae*, and to assign the first to *An. daciae*, since the two nucleotide positions that are considered the most stable species-specific diagnostic sites indicated that species (Brutsensov *et al.*, 2023) (Figure 4). Adults from that complex obtained from trappings were not submitted to molecular analyses. Our sequences were deposited in GenBank, accession numbers PP898970-PP898978 (Supplementary Table S1).

Citizen science data

Among the reports received by Mosquito Alert from Polish citizenship in 2023, five were possible EAMs. These reports were obtained in October-November 2023 from the Southern Kraków area (four reports from Dobczyce and one from Krosno) of which four were validated as 'probably *Ae. japonicus*' and the one from Krosno as 'probably *Cs. annulata*" by experts and/or AI (Figure 1 and Supplementary Table S2). By contrast to field data, these citizen science data provide no physical evidence, therefore results have to be interpreted. In the case of these reports, species identification is labelled as 'probably' and thus some uncertainty remains, specifically given the similarity of *Ae. japonicus* with *Ae. koreicus*. and of *Cs. annulata* with *Cs. subochrea* (Edwards, 1921).

Spatial distribution modelling of **Aedes japonicus**

Previous modelling of the environment suitability for *Ae. japonicus* did not suggest high probability of presence in the northern Czech Republic and southern Poland (Wint *et al.*, 2020). Unsurprisingly, the same modelling technique based on the presence/absence data set updated with VectorNet data to December 2023 plus our data suggest a high suitability in parts of Central and Eastern Europe. This model output (Figure 5) can serve as a base for selecting sampling locations that appear in red and yellow areas to be prioritised for field surveillance (e.g. Warsaw, south-east Poland, extreme south-west Ukraine).

4 Discussion

In the frame of VectorNet and its capacity building activities, we collected original mosquito distribution data in southern Poland and bordering areas of Czech Republic, Germany and Slovakia, in June and September – November 2023. Because of the suspected occurrence of *Ae. japonicus* in Poland, we targeted the exotic *Aedes* species in our sampling strategy, but we also collected

FIGURE 4 Alignment of our Polish ITS2 sequences (labels ending by 'PL') with sequences from Romania (RO; Nicolescu *et al.*, 2004), Belgium (BE; Smitz *et al.*, 2021), Luxembourg (LU; Schaffner *et al.*, 2023), and Russia (RU; Brusentsov *et al.*, 2023). Species-specific nucleotide positions are highlighted in blue for *Anopheles messeae*, in yellow for *An. daciae*.

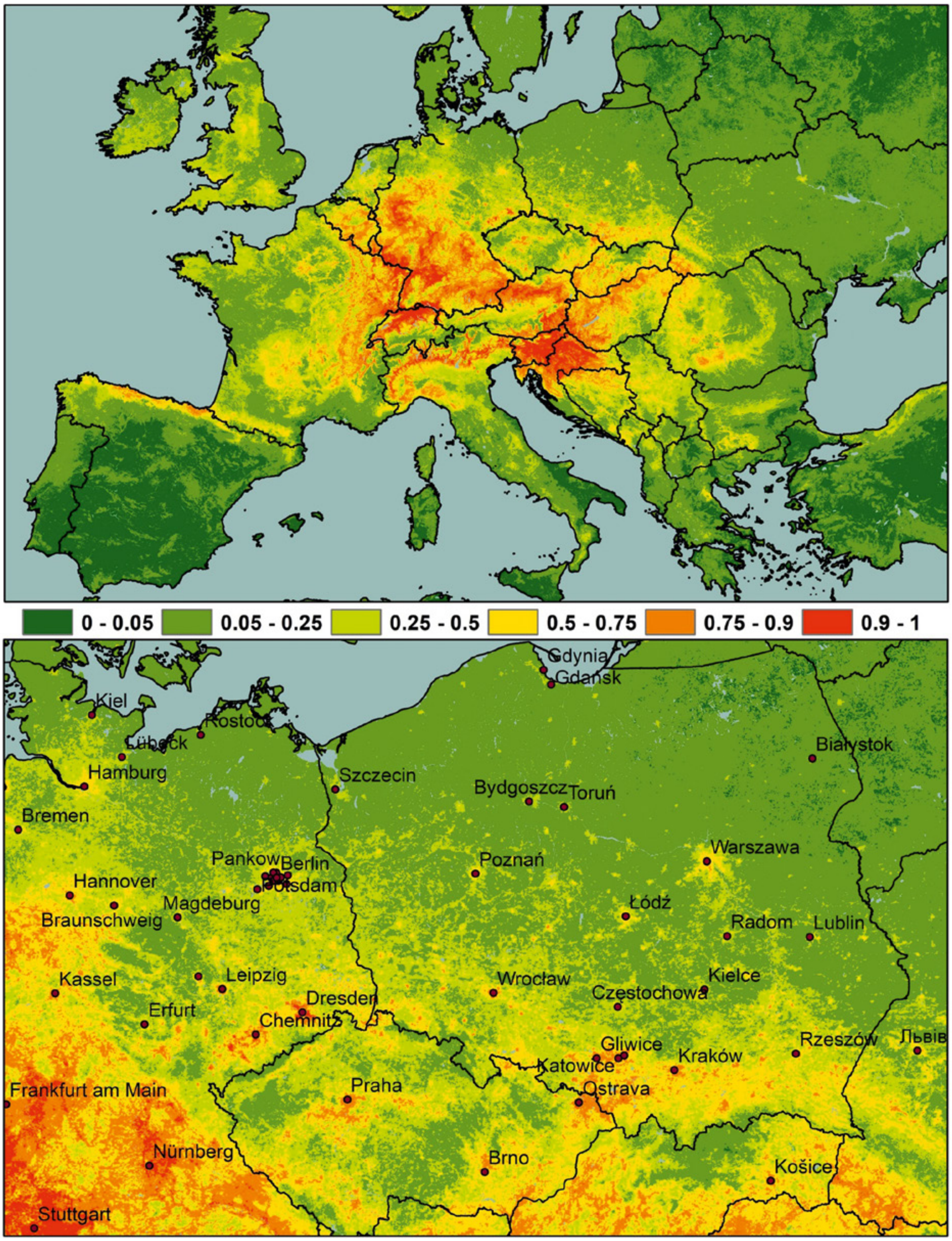

FIGURE 5 Predicted probability of presence of *Aedes japonicus* (updated from Wint *et al.* (2020)).

data on other mosquito species. We mainly collected mosquito immature stages from artificial and natural water containers (Figure 6A–D) but occasionally from other aquatic habitats (Figure 7A–B). During this snapshot field study, 22 mosquito taxa were detected. Our study provides the first substantiated records of *Ae. japonicus* and *An. petragnani* in Poland, while all other species collected were known to occur in the country (Lühken *et al.*, 2023; Robert *et al.*, 2019; Rydzanicz *et al.*, 2017).

For the native species we identified their presence in 127 species/NUTS3 combinations (113 for Poland), of which 94 are credited to the twelve taxa listed among the VectorNet priority species (Supplementary Table S5). These data will be used to update the pan-European vector distribution maps (https://www.ecdc.europa.eu/en/disease-vectors/surveillance-and-disease-data/mosquito-maps); the data collected in June 2023 is already included on these maps. Unsurprisingly, the species group formed by *Cx. pipiens* and *Cx. torrentium* was the most frequently encountered (found at 70 locations in total), followed by *An. plumbeus* (at 15 locations).

A photo posted on iNaturalist early 2022 suggested the occurrence of *Ae. japonicus* or *Ae. koreicus* at Bytom, southern Poland. Our survey provides evidence of the occurrence of the first species only: *Ae. japonicus* is

FIGURE 6 Examples of aquatic larval habitats found to yield mosquito species in Poland, 2023, from the category 'Human-made container' (A–D) and 'Natural container' (E). (A) Collection site of *Aedes japonicus*, *Anopheles petragnani* and *Culex pipiens*: various containers in community gardens at site PL043, Kielce, Kielecki, on 30/09/2023; (B) Collection site of *Ae. japonicus*, *Ae. geniculatus*, *Cx. pipiens* and *Culiseta annulata*: used tyres nearby a farm at site PL010, Pociekarb, Opolski, on 21/06/2023; (C) Collection site of *Ae. japonicus* and *Ae. geniculatus*: a plastic bucket in a cemetery at site PL005, Wałbrzych, Wałbrzyski, on 21/06/2023; (D) Collection site of *Ae. japonicus*: an iron barrel in a cemetery at site PL029, at Bielsko-Biała, Bielski, on 25/06/2023; (E) Collection site of *Ae. japonicus* and *Ae. geniculatus*: a tree hole (beech) at site PL027, Radziszów, Krakowski, on 25/06/2023.

FIGURE 7 Examples of aquatic larval habitats found to yield mosquito species in Poland, 2023, from the category 'Stagnant temporary water body' (A) and '(Semi-)permanent water body with vegetation' (B). (A) Collection site of *Anopheles claviger s.s.*, *Culex pipiens/torrentium* and *Culiseta annulata*: a temporary pond in a forest at site PL065, Krężnica Jara, Lubelski, on 03/10/2023. (B) Collection site of *An. claviger s.s.*, and *An. daciae*: a permanent marsh border at site PL065, Stadła, Nowosądecki, on 01/10/2023.

confirmed to occur at Bytom and was found at 21 locations in Poland, while *Ae. koreicus* was not detected. Also, no other EAM species such as the tiger mosquito *Aedes* (*Stegomyia*) *albopictus* (Skuse, 1894) was detected. However, considering the current spread of that species in Europe, including in countries neighbouring Poland, introductions into Poland by ground vehicles could certainly occur and its detection in the country is probable in the near future as long as surveillance is carried out (Medlock *et al.*, 2012; Wałęka *et al.*, 2023).

The invasive mosquito *Aedes japonicus* is clearly established over a large part of Poland (21 NUTS3 out of 73; approximately 80,500 km^2). Surprisingly the species was not found in a recent study of mosquitoes in cemeteries of Wrocław (Rydzanicz *et al.*, 2021), while we found the species at other locations south-west and north-east to that city, but at least 40 km away. We also found the species in the bordering Czech Republic, providing presence records from areas where it was previously not reported to occur (ECDC, 2022; Vojtíšek *et al.*, 2022a). By contrast, the finding in Germany confirms previous citizen science submissions from that area (Werner and Kampen, 2024, unpublished data). These occurrences suggest a possible spread of the species into Poland originating from the south, by active dispersal through forest corridors or passively via ground vehicle transport. This may have happened several years ago, at least before 2021 when a specimen was photographed at Bytom. However, it cannot be excluded that the species was introduced in the area via the importation of used tyres, since this remains a major pathway of EAM introduction into European countries (Ibañez-Justicia, 2020). Despite the observation of numerous locations that stayed negative for that species, it remains possible that the species already occurs in a larger area than described. Also, there is no obvious environmental or climatic reason that the species should not spread further northwards in Poland and eastwards into Ukraine, which might be one of the next European countries to be colonised by the species. There are indeed small habitats in that part of Central Europe which can be used to define the absence of *Ae. japonicus* (Wint *et al.*, 2020), and our modelling output suggests parts of Poland and Ukraine located outside the current know species' distribution range to be suitable to the species.

In Germany, *Ae. japonicus* was first found in 2008 (Schaffner *et al.*, 2009), and has since rapidly spread throughout the country (Koban *et al.*, 2019). There are few detections from the German area adjacent to Poland and no detections quite close to the Polish border, but this study suggests the species to occur in a much wider area on the German part of the country triangle Germany/Poland/Czech Republic than currently known. In the Czech Republic, the species was only recently found and like in Poland, the first indicative finding was submitted by a citizen in 2021. Subsequent field investigations showed the species to occur at three locations in the southern part of the country, while the centre and the north were not investigated (Vojtíšek *et al.*, 2022a). Our findings in the northern part suggest the species to occur all over the country. In Slovakia, we did not detect the species, but we investigated only a single location. Considering that the species was previously found in at least three geographically distant areas of Slovakia (Bratislava, Zvolen and Prešov) (Čabanová *et al.*, 2021), and that we demonstrated its presence at several locations in bordering Poland with the nearest finding located around 15 km from the border (Figure 1), we could expect *Ae. japonicus* to probably occur all over Slovakia. Overall, the species looks to be grossly underreported in Central Europe, and targeted surveys should be implemented in other parts of Poland and all over Central and (at least bordering) Eastern Europe to confirm this. Such a survey may also detect the possible introduction of other EAMs such as *Ae. koreicus* which is established in Germany (Hohmeister *et al.*, 2021) and was recently found in the Czech Republic (Vojtíšek *et al.*, 2022b) and in several locations of southern Slovakia (unpublished data). This is also the case for *Ae. albopictus* which is established in Germany (closest location: Berlin) and was found to have been introduced in both the Czech Republic and Slovakia (https://www.ecdc.europa.eu/en/publications-data/aedes-albopictus-current-known-distribution-october-2023), and it may only be a matter of time before the mosquito appears introduced at points of entry in Poland (Wałęka *et al.*, 2023).

Here some relevant citizen data were obtained from both iNaturalist and Mosquito Alert platforms. Even with a remaining level of uncertainty these provide valuable input. The first report (iNaturalist, 2021) motivated the performance of our field study in that region, while the others (Mosquito Alert) confirmed the occurrence of *Ae. japonicus* at some locations. In the latter, the uncertainty could be lifted since the presence of the species in the areas was evidenced by field data (Figure 1). The 3-tier human validation system provides high quality real-time data with unlimited scalability at a very low cost. The platform has provided several first detections at regional or national levels, such as the discovery of *Ae. japonicus* in Spain in 2018, more than 1,100 km away from the nearest European population (Eritja *et al.*, 2019). Thus, we suggest giving high priority in integrating the citizen contribution to the global mosquito surveillance scheme via platforms like iNaturalist or Mosquito

Alert as a trigger to target field monitoring towards highest-probability areas, as well as to engage citizens in social action on mosquitoes, disease prevention and public health affairs. The Mosquito Alert has the asset to establish an alert system directly linked to national Public Health agencies (although no formal relationship is established yet in the case of Poland). So, it serves as a real-time early-warning system for public authorities, keeping an internal distribution map to automatically emit alerts of new detections – which was the case for Poland – whereas iNaturalist needs to be manually checked for specific occurrences. The detection of introduced tiger mosquito populations by citizens could also be a relevant target of citizen science-based passive mosquito surveillance. This would enhance the formal mosquito surveillance scheme and have no costs other than promoting the system to the public, which can be done through a variety of low-cost information channels.

5 Conclusions

Our findings imply changes in threats to public and veterinary health for Central Europe. While potential vector species of West Nile and Rift Valley fever viruses are already present in Poland (Kwaśnik *et al.*, 2021; Robert *et al.*, 2019; Vogels *et al.*, 2017; Wint *et al.*, 2020), the establishment of *Ae. japonicus* extends the putative vector species list. Although this species has not proven yet to be an efficient vector of these arboviruses under natural conditions, *Ae. japonicus* has the potential to contribute to their transmission since it has shown to be competent in the laboratory to both viruses (among others) and was found naturally infected by West Nile virus on a number of occasions in the US (De Carlo *et al.*, 2020; Medlock *et al.*, 2015; Schaffner *et al.*, 2013; Vogels *et al.*, 2017) and thus facilitate outbreaks in areas where it is abundant, given the viruses are introduced.

Supplementary material

Supplementary material can be found online at https://doi.org/10.6084/m9.figshare.26236307

Figure S1. Photo posted on iNaturalist platform on 02/01/2022 and taken at Bytom, 13/10/2021, with mosquito adult female identified as *Aedes japonicus/koreicus*.

Table S1. List of *Anopheles* specimens with ITS2 sequences deposited to GenBank and results of molecular taxonomic assignment, collection site and date.

Table S2. Data from Mosquito Alert (MA) reports and species diagnostics for Poland, 2023.

Table S3. Detailed sampling effort per admin units in our snapshot mosquito field study in Central Europe, 2023.

Table S4. Number of locations positive for a mosquito species per admin unit in our snapshot mosquito field study in Central Europe, 2023.

Table S5. Number of species/NUTS3 combinations in our snapshot mosquito field study in Central Europe, 2023.

Table S6. Detailed field sampling data of our snapshot mosquito field study in Central Europe, 2023.

Acknowledgements

We are grateful to Weronika Gołofit for technical assistance, to Alexander Weigand for contributing to the molecular analyses, to Adrian Tync for providing details about the finding of an *Ae. japonicus*-like mosquito at Bytom, Poland, and to all Mosquito Alert citizen contributors. We highly acknowledge Andrzej Kruk, Dean of the Faculty of Biology and Environmental Protection, University of Łódź, Poland, for hosting the capacity building training, and we thank Marieta Braks for coordinating VectorNet. The funding of this study was provided by VectorNet and the specific contract #4 of the European Food Safety Authority, implementing framework contract No ECDC/2019/020.

Conflict of interest

Francis Schaffner is editor-in-chief of the Journal of the European Mosquito Control Association; he had no influence in the review process and decision making on this manuscript. The other co-authors declare no conflict of interest.

Data availability

The detailed data that supports the findings of this study are available in the supplementary material Table S6 of this article.

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