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Crossing continents: First record and evidence of establishment of the Australian backyard mosquito Aedes notoscriptus (Diptera: Culicidae) in Europe, Bosnia and Herzegovina

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Abstract

Background

Mosquitoes (Diptera: Culicidae) are important vectors of human and animal pathogens, while globalization, climate change, and human mobility increasingly facilitate the spread of non-native species. Invasive *Aedes* mosquitoes are particularly successful due to their ecological plasticity, use of artificial containers, and desiccation-resistant eggs, which enable passive long-distance dispersal. *Aedes notoscriptus*, a medically and veterinary important mosquito native to the Australasian region, has established populations beyond its native range, but until now had not been documented as established in Europe or elsewhere in the Palearctic region. Here, we report the first detection and evidence of establishment of *Ae. notoscriptus* in Bosnia and Herzegovina, Europe, and the Palearctic, supported by morphological and molecular identification.

Methods

Mosquito sampling was conducted southern Bosnia and Herzegovina, from September 2024 to June 2026 using CO_2-baited CDC traps and larval collections. After the first confirmation of *Ae. notoscriptus* in Mostar, targeted study was expanded to surrounding sites to assess its local distribution. Specimens were identified morphologically and confirmed molecularly by sequencing the mitochondrial cytochrome c oxidase subunit I (COI) barcode region. The sequences obtained were compared with GenBank and BOLD reference data, and genetic relationships were assessed using phylogenetic and haplotype analyses.

Results

Aedes notoscriptus was first sampled in Mostar in September 2024 and was repeatedly recorded at the same site in 2025 and 2026, and additionally surrounding localities in 2026. In total, 86 specimens were collected from eight localities, including larvae found near the initial detection site, providing evidence of local establishment. COI sequencing of nine specimens confirmed their identity as *Ae. notoscriptus*, with all sequences assigned to BOLD BIN ADM7085. The Bosnia and Herzegovina specimens comprised two closely related haplotypes, also represented among reference sequences from Australia and New Zealand, and phylogenetic analysis placed them within the same major *Ae. notoscriptus* lineage.

Conclusions

The detection of *Ae. notoscriptus* in Bosnia and Herzegovina represents the first documented record of the species in Europe and provides evidence of an established, reproducing population. Its repeated

detection, local spread, and ecological adaptability underscore the need for continued surveillance to determine its distribution, potential for further expansion, and medical and veterinary significance.

Background

Mosquitoes (Diptera: Culicidae) are among the most important vectors of human and animal pathogens worldwide [1, 2]. In recent decades, their geographical distribution has changed significantly due to globalization, international trade, climate change, human mobility, land-use change, and urbanization, facilitating the movement and establishment of mosquito species beyond their native ranges [3–5]. The Western Palearctic encompasses Europe and adjacent regions and forms part of the broader Palearctic biogeographical region [6–8]. Mosquito distribution within this region is strongly influenced by climatic and ecological conditions, including temperature, precipitation, and the availability of suitable aquatic habitats for larval development, vegetation cover, and anthropogenic factors [9, 10]. In Europe, increasing human-mediated movement and trade have facilitated the introduction of non-native mosquito species, while suitable climatic and ecological conditions have enabled their subsequent establishment and spread. The Western Palearctic supports a diverse mosquito fauna, including numerous species of medical and veterinary importance [9, 10].

Invasive *Aedes* mosquitoes are particularly successful because they can to readily exploit small artificial water-holding containers and produce desiccation-resistant eggs. Following embryonic development, these eggs can remain viable during prolonged dry periods and hatch after subsequent flooding, facilitating both survival under unfavorable environmental conditions and passive long-distance dispersal through human-mediated transport of artificial containers and traded goods [11–13]. Four *Aedes* species are currently considered invasive and established in parts of Europe: *Aedes albopictus* (Skuse, 1894), *Aedes aegypti* (Linnaeus, 1762), *Aedes japonicus japonicus* (Theobald, 1901), *and Aedes koreicus* (Edwards, 1917), *while Aedes atropalpus* (Coquillett, 1902*)* has also been recorded in Europe but is not currently considered an established invasive species [14]. *Aedes albopictus* was first detected in Europe in Albania in 1979 and subsequently became established in Italy and other European countries [15]. *Ae. japonicus* and *Ae. koreicus* subsequently established populations in several European countries [14]. *Aedes aegypti*, after disappearing from most of Europe during the twentieth century, became established on Madeira following its detection in 2005 and was subsequently re-established in Cyprus, where overwintering was confirmed in 2022 [16, 17].

Aedes notoscriptus (Skuse, 1889), commonly known as the Australian backyard mosquito, is native to the southwestern Pacific region and is widely distributed across Australia and several neighboring islands, including Papua New Guinea, the Solomon Islands, New Caledonia, and Indonesia [18, 19]. Although *Ae. notoscriptus* was originally associated with temperate and tropical forest ecosystems, where its immature stages develop in natural water-holding habitats such as tree holes, bamboo stumps, leaf axils, and rock pools, the species has demonstrated considerable ecological plasticity and successfully colonized urban and suburban environments [19]. In these environments, larvae readily develop in a wide range of artificial water-holding containers, contributing to the species becoming a

common peridomestic mosquito across much of its native range [20, 21]. Substantial mitochondrial genetic differentiation has been reported among *Ae. notoscriptus* populations, indicating the presence of divergent lineages within the taxon [22]. Its close association with human settlements has facilitated repeated introductions beyond its native range. It was introduced into New Zealand in the early twentieth century [23] and was first detected in southern California in 2014, where it subsequently expanded geographically [19]. Before the present study, no established population of *Ae. notoscriptus* had been reported in Europe or elsewhere in the Palearctic.

In addition to its invasive potential, *Ae. notoscriptus* is of considerable medical and veterinary importance. In Australia, it is an important vector of the canine heartworm *Dirofilaria immitis* [24–26] and has been implicated in the transmission of Ross River virus and Barmah Forest virus [27, 28]. Experimental studies have also demonstrated vector competence of *Ae. notoscriptus* for several other medically important arboviruses, including chikungunya, yellow fever viruses Zika, West Nile, and Japanese encephalitis [29–33]. Its expanding non-native distribution, ecological adaptability, and demonstrated vector competence underscore the importance of continued surveillance and early detection in regions where establishment may occur.

This study reports the first detection and evidence of establishment of *Ae. notoscriptus* in Bosnia and Herzegovina, in Europe, and in the Palearctic. We provide morphological and molecular confirmation of species identity, compare the detected COI haplotypes with the currently available global sequence dataset, and discuss the ecological significance and potential implications of these findings for future surveillance, vector control, spread, and possible invasive behavior.

Materials and methods

Study area

The study was conducted in Herzegovina (Fig. 1), the southern region of Bosnia and Herzegovina, which has a Mediterranean climate with hot, dry summers and mild, wet winters. Mean daily maximum temperatures reach approximately 32–34°C during July and August, while winter mean daily maximum temperatures range from 9 to 11°C. Annual precipitation averages around 1,300–1,500 mm, with most rainfall occurring in late autumn and winter and a pronounced dry period during summer [34]. Such climatic conditions favor the persistence of a wide range of mosquito breeding habitats and support high seasonal mosquito abundance, particularly during the warm period of the year. The region consists predominantly of karst landscapes interspersed with urban and suburban settlements, where numerous artificial water-holding containers provide suitable breeding habitats for invasive *Aedes* mosquitoes. Herzegovina is also situated along major transport routes connecting the Adriatic coast with the inland Balkan Peninsula, increasing the likelihood of accidental introductions of non-native mosquito species [35, 36].

Within Herzegovina, the first detection of *Ae. notoscriptus* was recorded in Mostar, a city of 105,797 inhabitants [37], located at an altitude of 60–80 m above sea level in a valley traversed by the Neretva River and its tributaries [38]. It has a typical Mediterranean climate, with long, hot summers and mild to moderately cold winters. Mostar is widely known as one of the warmest cities in the region and usually has a high number of sunny days throughout the year [39].

Mosquito collection and morphological identification

As part of an entomological study on the fauna and ecology of mosquitoes in Herzegovina, adult and larval mosquitoes were sampled in various parts of Herzegovina from September 2024 to June 2026 (geographic coordinates and description of the locality can be found in Additional file 1: Table 1). Sampling was conducted once a month from April to October, with 2024 serving as a preliminary research period and 2025 and 2026 as the full study period.

Table 1

Occurrence of *Ae. notoscriptus*, including collection sites, number of specimens collected and descriptions of habitats. The mark"-" indicates that no breeding sites were found in the vicinity of the associated locations. Site no.: site numbers correspond to the numbered sampling locations shown in Fig. 1.

Site no.	City district	Month/Year	Habitat	GPS coordinates	No. of adults	No. of larvae
1	Mostar	September/ 2024	Garden with abundant vegetation; vegetables, fruit trees, and weeds; partially sunny.	43.332714 17.818687	1	-
		September/ 2025			3	-
		May/2026			5	0
		June/ 2026			31	2
2	Luka 1	June/ 2026	Garden with abundant vegetation; vegetables; shaded.	43.333149 17.818241	10	-
3	Luka 2	June/ 2026	Garden with abundant vegetation; vegetables, fruit trees; near a fertilizer bucket; partially sunny.	43.333052 17.817570	7	-
4	Tekija	June/ 2026	Garden with abundant vegetation; vegetables, fruit trees; partially sunny.	43.327639 17.820919	1	-
5	Šehovina 1	June/ 2026	Yard in front of the house with moderate vegetation, mostly flowers in pots, partially sunny.	43.334994 17.821092	1	-
6	Šehovina 2	June/ 2026	Sunny front yard with lots of flowers and grass.	43.337272 17.806934	2	-
7	Mahala	June/ 2026	Front yard with moderate vegetation, mostly grass and a small orchard, sunny.	43.329967 17.815708	1	-
8	Franjevačka street	June/ 2026	Yard in front of the building with moderate grass and tree vegetation; the Radobolja River flows nearby; partially sunny.	43.331248 17.822082	22	-
9	Mahala 2	June/ 2026	Front yard with fruit trees, flowers, and grass, partially sunny.	43.333757 17.815404	0	-

Site no.	City district	Month/Year	Habitat	GPS coordinates	No. of adults	No. of larvae
10	Mahala 3	June/ 2026	Sunny front yard with fruit trees and flowers.	43.335057 17.813858	0	-
11	Cernica	June/ 2026	Yard near the Radobolja River with lots of flowers and trees, partially sunny.	43.338009 17.810161	0	-

After molecular confirmation of *Ae. notoscriptus* at the Mostar site, a targeted delimitation study was initiated in June 2026. Sampling was expanded by deploying 10 additional CDC traps within radii of 500 m and 1,000 m from the original detection site (Fig. 1, red and orange dots, Table 1). The additional sampling sites were located in densely populated urban residential areas with abundant vegetation, including fruit trees, vegetable gardens, ornamental plants, weeds, and other trees (Table 1).

Adult mosquitoes were collected using CO_2-baited CDC traps, with dry ice used as the CO_2 source and one trap deployed at each sampling site. Traps were operated overnight for approximately 11–14 h (deployed between 18:00 and 20:00 and retrieved between 07:00 and 08:00 the following morning). Larvae were collected with a dipper around the trapping localities from selected breeding sites suspected of harboring invasive *Aedes* mosquitoes.

Specimens were identified morphologically according to Becker et al. [10], pooled by species, location, and sampling date, and stored at − 20°C for molecular analysis. In addition, we retained one individual of each species to create a reference mosquito collection of the mosquito fauna of Herzegovina (stored at the Veterinary Institute of Herzegovina-Neretva Canton, Bosnia and Herzegovina). Specimens were photographed with an Olympus DP25 digital camera (Olympus, Tokyo,Japan) mounted on an Olympus SZX16 stereomicroscope (Olympus, Tokyo, Japan).

Molecular identification

Genomic DNA was extracted from individual adult mosquito legs and whole larvae using the GenElute™ Mammalian Genomic DNA Miniprep Kit (Sigma, Taufkirchen, Germany). The manufacturer's rodent tail protocol was followed with minor modifications, including overnight incubation with proteinase K and elution of DNA in 100 µl of elution buffer. The mitochondrial cytochrome c oxidase subunit I (COI) barcode region [40] was amplified using the universal primers LCO1490 and HCO2198 [41]. The PCR products were sequenced bidirectionally at Macrogen Inc. (Amsterdam, The Netherlands) using the amplification primers.

Data analysis

Consensus sequences of the mitochondrial COI marker were manually inspected, edited, and aligned in BioEdit v7.2.5 [42]. The finalized sequences were subsequently submitted to the NCBI GenBank database and the Barcode of Life Data System (BOLD) (GenBank accession numbers QB039506 - QB039514, and BOLD ID numbers: CUBIH001 – CUBIH009). Sequences were inspected to confirm the absence of stop codons, insertions, and ambiguous nucleotide positions. Sequence identity and taxonomic assignment were initially assessed using BLASTn searches against the NCBI GenBank database [43] and then verified using reference sequences available in the Barcode of Life Data System (BOLD; accessed September 29, 2026). At the time of analysis, BOLD records assigned to *Ae. notoscriptus* were associated with five distinct Barcode Index Numbers (BINs).

Reference sequences retrieved from GenBank and BOLD and included in subsequent genetic analyses are listed in Additional file 2: Table 2 including their accession numbers or BOLD Process IDs, available geographic information, and BIN assignment.

Table 2
Specimen metadata and molecular identification data for *Aedes notoscriptus* collected at Mostar, from initial detection site (Mostar), including GenBank accession numbers (GenBank an) and BOLD records.

GenBank an	BOLD ID	Field ID	Sequence name	Stage[a]	Date collected
QB039506	CUBIH001	MO6-24	*Aedes notoscriptus*_6_ BIH	I	September/ 2024
QB039507	CUBIH002	MO10-25	*Aedes notoscriptus* _10_ BIH	I	September/ 2025
QB039508	CUBIH003	MOan1-26	*Aedes notoscriptus* _an1_ BIH	L	June/ 2026
QB039509	CUBIH004	MOan2-26	*Aedes notoscriptus* _an2_ BIH	I	June/ 2026
QB039510	CUBIH005	MOan3-26	*Aedes notoscriptus* _an3_ BIH	I	June/ 2026
QB039511	CUBIH006	MOan4-26	*Aedes notoscriptus* _an4_ BIH	I	June/ 2026
QB039512	CUBIH007	MOan5-26	*Aedes notoscriptus* _an5_ BIH	I	May/ 2026
QB039513	CUBIH008	MOan6-26	*Aedes notoscriptus* _an6_ BIH	I	May/ 2026
QB039514	CUBIH009	MOan7-26	*Aedes notoscriptus* _an7_ BIH	I	May/ 2026

[a]I-imago, female; L-larva

Intraspecific genetic divergence within *Ae. notoscriptus* was analyzed using pairwise genetic distances calculated in MEGA 11 using the p-distance method [44]. Phylogenetic relationships among the analyzed samples were reconstructed using the maximum-likelihood (ML) method implemented in IQ-TREE 2 [45], with *Ae. albopictus* and *Ae. japonicus* included as outgroup taxa. The best-fit nucleotide substitution model was selected using ModelFinder [46]. Branch support was assessed using the SH-like approximate likelihood ratio test (SH-aLRT; [47]) and ultrafast bootstrap approximation (UFBoot2; [48]) each based on 1000 replicates. Phylogenetic trees were visualized and refined in FigTree v1.4.3 [49]. A haplotype network was generated in PopART v1.7 [50] using the TCS algorithm. Final figures, including both the phylogenetic tree and haplotype network, were assembled and graphically edited in Inkscape v1.4.2.

Results

The first record of *Ae. notoscriptus* was obtained in September 2024 during entomological study on the fauna and ecology of mosquitoes in Herzegovina. A single specimen captured with CO_2-baited CDC traps in the historic city center of Mostar (43°19'58.071" N, 17°49'07.150" E; Fig. 1, site 1). At the time of collection, the specimen could not be reliably identified using the available morphological identification key for European mosquito species [10], as its morphological characteristics did not correspond to any species included in the key.

During subsequent sampling in 2025, three additional specimens with the same morphological characteristics were collected at the same location, followed by five additional specimens in May 2026 (Table 1). During targeted sampling in June 2026, 75 adults of *Ae. notoscriptus* were collected from seven of the ten additional sampling sites. Two larvae were also collected in the immediate vicinity of the initial detection site. This site was a densely vegetated private garden behind a residential house. Vegetation included ornamental plants, fruit trees, and other cultivated plants, providing suitable habitat for container-breeding mosquitoes (Fig. 2). The larvae were collected from a water-filled container with a large amount of decomposing leaves beneath a pomegranate tree. The water temperature at the time of collection was 26°C. Both larvae were identified morphologically, and one was subsequently confirmed molecularly (Table 2).

In total, 86 specimens of *Ae. notoscriptus* were collected from eight localities and morphologically identified. Nine specimens from the initial Mostar site were included in the molecular analyses (Table 2). Analysis based on the COI marker confirmed their identification as *Ae. notoscriptus* with all sequences assigned to BIN BOLD:ADM7085. The sequences showed 100% identity to several sequences from Australia and New Zealand, while a shorter sequence from the United States showed no nucleotide differences across the overlapping region (see Additional file 2).

Following molecular confirmation, the specimens were reexamined using the determination characters for adult females of the Australian species described by Becker et al. [10], and were further verified with an online identification key according to Gyawali et al. [51]. The observed morphological characters were

consistent with the diagnostic features of *Ae. notoscriptus* (Fig. 3), as described below. The specimens were dark-colored, medium-sized mosquitoes with a characteristic pattern of pale scales on the body. The scutum displayed a distinctive lyre-shaped pattern formed by silvery white to golden scales. The proboscis bore a narrow pale median ring, and the legs were dark with conspicuous white rings on the tarsi and pale markings on the femora and tibiae. The abdomen was dark, with white basal bands and lateral patches of pale scales on the tergites (Fig. 3).

Genetic distances

Pairwise genetic distance analysis revealed very low sequence divergence among the nine *Ae. notoscriptus* specimens collected in Bosnia and Herzegovina. Genetic distances among the nine study sequences ranged from 0 to 0.00159 substitutions per site. The sequences formed two closely related haplotypes; five specimens shared an identical sequence, whereas the remaining four shared a second haplotype differing from the first by approximately 0.16%. All nine Bosnia and Herzegovina sequences were assigned to BIN BOLD:ADM7085.

In comparisons between the nine Bosnia and Herzegovina study sequences and reference sequences retrieved from public databases, pairwise genetic distances ranged from 0.000 to 0.0635 substitutions per site. The first haplotype was identical across the analyzed COI region to reference sequences GBAAX55423-24 and MT880025.1 from New Zealand and GBDP16992-15, KU495078.1, and MOAV075-15 from Australia, whereas the second haplotype was identical to GBAAZ8357-24 from Australia and MT880024.1 from New Zealand. The shorter reference sequence from the United States (CASBI23589-26) showed no nucleotide differences from either haplotype across the overlapping region. The highest genetic distance involving the Bosnia and Herzegovina study sequences was 0.0635 substitutions per site and was observed between the second haplotype and reference sequences GBMNA9928-19 and GBMTG3575-16, which were assigned to another BOLD BIN. When all 53 sequences included in the p-distance analysis were considered, the maximum genetic distance was 0.0715 substitutions per site. At the time of analysis, *Ae. notoscriptus* was represented by five distinct BINs in BOLD. Overall, the low genetic divergence among the Bosnia and Herzegovina specimens and their close similarity to confirmed reference sequences support their molecular identification as *Ae. notoscriptus*.

Phylogenetic analysis

Maximum-likelihood analysis of COI sequences placed all specimens from Bosnia and Herzegovina within a well-supported *Ae. notoscriptus* COI clade recovered in the present dataset, clearly separated from the outgroup taxa (*Ae. japonicus* and *Ae. albopictus*). Within this clade, which corresponds to the BIN BOLD:ADM7085, the Bosnian and Herzegovina specimens did not form a distinct lineage but were interspersed among reference sequences from Australia and New Zealand (Fig. 4). Notably, the reference sequence of a single specimen from the USA was also included in this clade.

The TCS haplotype network based on COI sequences (Fig. 5) showed that the *Ae. notoscriptus* specimens from Bosnia and Herzegovina belong to two closely related haplotypes already detected in populations from Australia (Australian Capital Territory, Victoria, and New South Wales) and New Zealand. Both haplotypes are embedded within the overall network and differ by only a few mutational steps from several other haplotypes previously reported from across Australia and New Zealand.

Discussion

This study provides the first documented record of *Aedes notoscriptus* in Europe and evidence of an established, reproducing population in Bosnia and Herzegovina. The species was repeatedly detected from 2024 to 2026, initially in the Mostar city center and subsequently in multiple districts across the surrounding urban area. The detection of larvae provides direct evidence of local reproduction, while repeated occurrence over successive years, including two winter periods, indicates population persistence.

Nevertheless, the actual timing of introduction and the possible point of entry remain unknown, as the first detection in 2024 does not necessarily represent the time of initial arrival, and the detection site itself does not provide information on the potential pathway of introduction. The increasing number of positive localities around the initial detection site is consistent with local spatial expansion. Active dispersal of *Ae. notoscriptus* is generally limited, with mean dispersal distances of approximately 105–180 m and reported maxima below 250 m [52, 53]. The observed pattern may therefore reflect gradual local spread from established breeding sites, similar to the continued geographical expansion documented following the introduction of *Ae. notoscriptus* in New Zealand [54] and southern California [19]. Continued surveillance will be necessary to determine the current extent of the population and any further expansion.

The occurrence of this species, which predominantly inhabits the Australasian region, in the Western Palaearctic represents a significant range expansion and aligns with the broader pattern of human-mediated dispersal observed in invasive container-breeding *Aedes* mosquitoes. Several species, including *Ae. albopictus*, *Ae. japonicus*, and *Ae. koreicus*, have established themselves in Europe following human-assisted introduction and spread [4, 15, 55]. A chronological overview of selected *Aedes* species recorded in Europe, including their native ranges, likely introduction pathways, establishment status, and invasive potential, is provided in Additional file 3: Table S3[4, 15, 19, 56–69]. Transport of artificial water-holding containers and other goods may represent plausible pathways for *Ae. notoscriptus* [19], particularly because its desiccation-resistant eggs can remain viable for extended periods [13]. Adults can also survive the conditions of air transport, indicating aircraft as an additional potential pathway for passive dispersal [70]. However, the present data provide no evidence for any specific introduction route, and neither the geographic origin nor the pathway of introduction can currently be resolved.

The larval habitat observed in Mostar is consistent with the ecological plasticity of *Ae. notoscriptus*. The species occupies both natural and artificial water-holding habitats and is closely associated with

residential and urban environments [19]. In introduced populations, larvae are commonly found in domestic containers and other small water-holding habitats [19, 71]. The shaded, water-filled container with decomposing plant material in which larvae were collected in Mostar therefore represents a typical breeding habitat and suggests that urban and peri-urban environments in the region may provide suitable conditions for the species' persistence.

The Mediterranean-influenced climate of southern Herzegovina may also be compatible with the climatic tolerance of *Ae. notoscriptus*. The species occurs across a broad climatic range in Australia and has established in temperate New Zealand [54], while experimental studies indicate that larval development has been reported across approximately 15–29°C [72]. Although the repeated detections indicate that the population can persist locally, broader climatic suitability and potential distribution in Bosnia and Herzegovina cannot yet be inferred and require further surveillance.

Molecular analyses independently confirmed the morphological identification and provided additional information on the genetic characteristics of the Bosnia and Herzegovina population. BLAST and BOLD analyses showed up to 100% sequence identity with reference *Ae. notoscriptus* sequences, and all nine Bosnia and Herzegovina specimens were assigned to the same BOLD BIN. Additional BINs associated with *Ae. notoscriptus* include specimens from Australia, consistent with the substantial mitochondrial structuring and deeply divergent genetic lineages previously reported within the species [22].

The Bosnia and Herzegovina specimens showed very low mitochondrial variation in the analyzed COI region, comprising two closely related haplotypes with a maximum genetic distance of 0.00159 (approximately 0.16%), whereas pairwise distances among reference sequences in the complete dataset reached 0.0715 (7.15%). The maximum-likelihood phylogeny and haplotype network showed the same general pattern: the Bosnia and Herzegovina specimens did not form a distinct or highly divergent European mitochondrial lineage, but clustered with previously characterized *Ae. notoscriptus* sequences, with identical COI sequences occurring in geographically distant populations from Australia and New Zealand, and a shorter sequence from the United States within the same BOLD BIN. These available mitochondrial data therefore do not allow the geographic origin or introduction pathway of the Bosnia and Herzegovina population to be determined. Higher-resolution nuclear or genome-wide markers, combined with broader geographical sampling, would be required to reconstruct the introduction history more precisely.

Repeated detection of *Ae. notoscriptus* over three consecutive years, including two intervening winters, together with the detection of larvae, provides strong evidence of an established, reproducing population and local persistence under the climatic conditions of Herzegovina. Multi-year persistence has likewise been reported in the established southern California population [19]. Although the overwintering stage in Bosnia and Herzegovina remains unknown, *Ae. notoscriptus* has been reported to overwinter as larvae and adults in cooler temperate regions of New Zealand [19, 23]. Its desiccation-resistant eggs may provide an additional mechanism for persistence under unfavorable conditions, with a proportion remaining viable after approximately one year of dry storage under experimental conditions [13].

The medical and veterinary importance of *Ae. notoscriptus* warrants attention because of its known vector potential. In Australia, this species has been associated with transmission of Ross River virus (RRV) and Barmah Forest virus (BFV) [28, 73]. Experimental studies have also demonstrated susceptibility and/or transmission competence for chikungunya, yellow fever, and Zika, West Nile, Japanese encephalitis viruses [29–33]. In addition, *Ae. notoscriptus* is recognized as an important domestic vector of dog heartworm, *Dirofilaria immitis*, with substantial vector efficiency [24–26]. Since *D. immitis* has also been documented in dogs in Bosnia and Herzegovina [74], the coexistence of a competent mosquito vector and a locally present parasite supports the inclusion of veterinary surveillance in future assessments.

These findings highlight the need for continued surveillance of *Ae. notoscriptus* in Bosnia and Herzegovina. Delimitation surveys and repeated seasonal sampling should clarify the current geographical extent, abundance, phenology, reproductive activity, and overwintering of the population. Surveillance should include both immature and adult stages, with particular attention to artificial water-holding containers in urban and peri-urban environments, in line with European guidance for newly introduced invasive mosquitoes [75]. Pathogen-oriented and veterinary surveillance would also help assess the medical and veterinary relevance of the population.

Experimental and field studies indicate that container-targeted control can reduce *Ae. notoscriptus* populations [76]. Early management should therefore focus on source reduction and larval habitat management, approaches that are also central to control programs for other invasive *Aedes* species [77]. Continued surveillance combined with targeted control, where necessary, will be important for assessing and limiting the further spread and potential health impact of this newly recorded mosquito species in Europe.

Conclusion

This study documents the first record of *Ae. notoscriptus* in Europe and provides evidence of an established, reproducing population in Bosnia and Herzegovina. Repeated detections over three consecutive years, the presence of larvae, and records from multiple nearby localities indicate local persistence and ongoing spatial expansion. This represents a substantial extension of the species' known geographical range and illustrates the capacity of an ecologically adaptable, container-breeding mosquito to overcome major biogeographic barriers, most likely through human-mediated dispersal. Its ecological plasticity, use of artificial breeding habitats, and ability to persist under the climatic conditions of southern Herzegovina may facilitate further establishment and spread. Continued entomological, molecular, and pathogen-focused surveillance will be essential to determine whether the population remains localized around Mostar or marks the beginning of a wider expansion within Bosnia and Herzegovina and potentially elsewhere in Europe, while also clarifying its overwintering strategy, introduction history, and potential medical and veterinary significance.

Declarations

Ethics approval and consent to participate:

Not applicable.

Consent for publication:

Not applicable.

Competing interests:

The authors declare that they have no competing interests.

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Author Contribution

L.P.H., E.M., and N.B. conceived and designed the study. L.P.H. and E.M. conducted field sampling. L.P.H., E.M., N.B., and I.V. performed morphological identification. L.P.H. and N.B. performed the molecular analyses. N.B., B.B.M., and G.V. contributed to data analysis and interpretation. G.V. assisted with the technical editing of the figures. N.B. drafted the manuscript. N.K., N.T., and A.Z. critically reviewed and edited the manuscript. All authors read and approved the final version of the manuscript.

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During the preparation of this manuscript, the authors used Gemini Notebook and InstaText for literature review and grammatical improvement of the text. The graphical abstract was prepared with the assistance of ChatGPT (OpenAI) using original mosquito photographs and geographic information provided by the authors. The authors reviewed and edited all AI-assisted output and take full responsibility for the content of this publication.

Data Availability

The datasets generated and/or analyzed during the current study are included in this published article and its additional files. Sequence data have been submitted to GenBank under accession numbers QB039506–QB039514 and to BOLD within project CUBIH under Process IDs CUBIH001–CUBIH009; these identifiers are also provided in Additional file 2. The complete dataset of aligned sequences used for genetic distance, phylogenetic and haplotype analyzes is available upon request.

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Figures

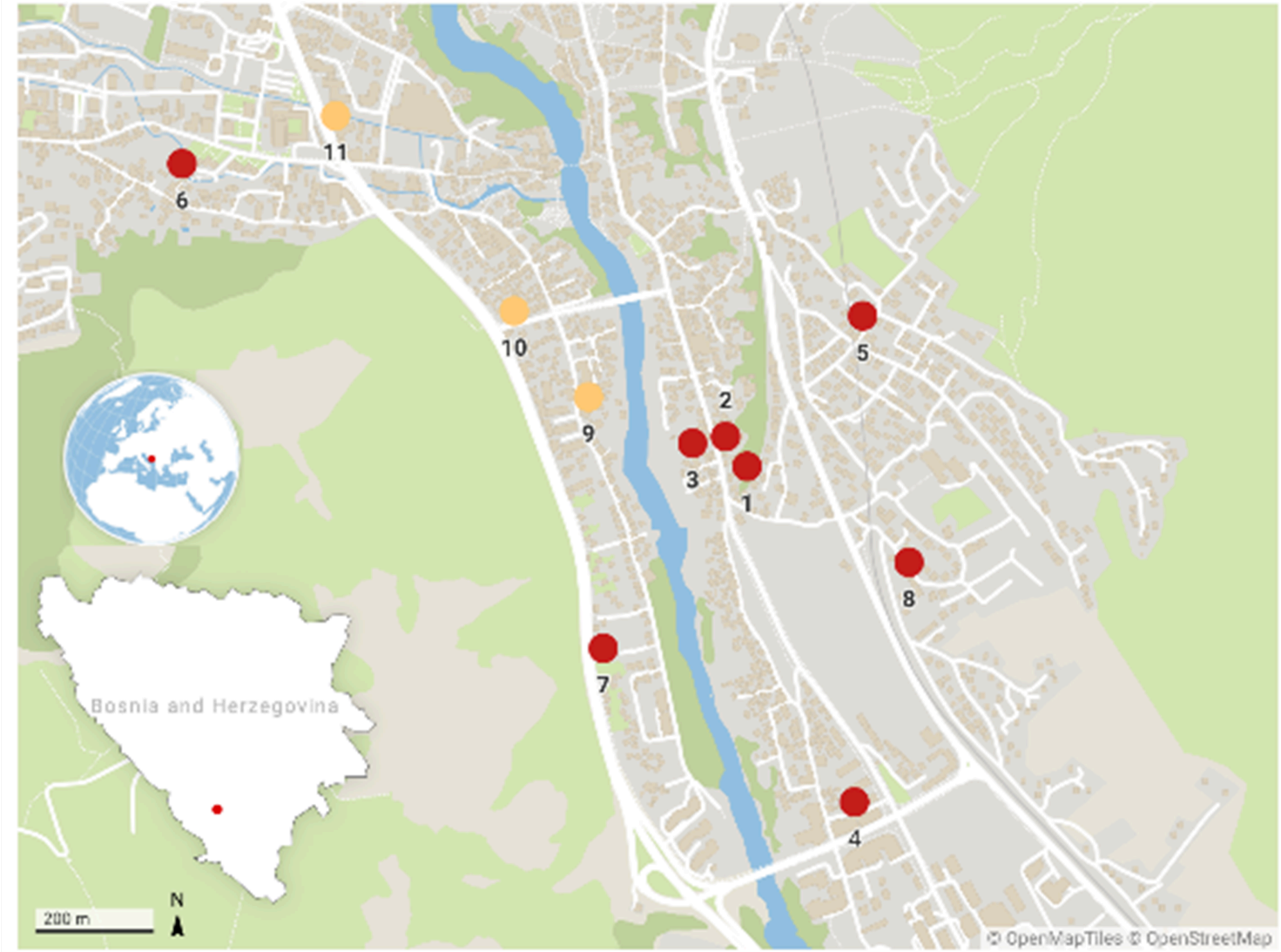


Figure 1

Sampling area across the city of Mostar, with locations surveyed in 2026. Red dots indicate the presence of *Ae. notoscriptus*specimens, while orange dots indicate their absence. Site number one is also the initial detection site of *Ae. notoscriptus*.



Figure 2

Habitat characteristics at the initial detection site of *Ae. notoscriptus* (a), and the habitat where *Ae. notoscriptus* larvae were found (b).



Figure 3

The adult female of *Ae. notoscriptus* from the Mostar location, sampled in 2026. Red markers indicate selected diagnostic morphological characters, including the characteristic lyre-shaped pattern of pale

scales on the scutum, pale abdominal markings, and distinct pale bands and markings on the legs and tarsi.

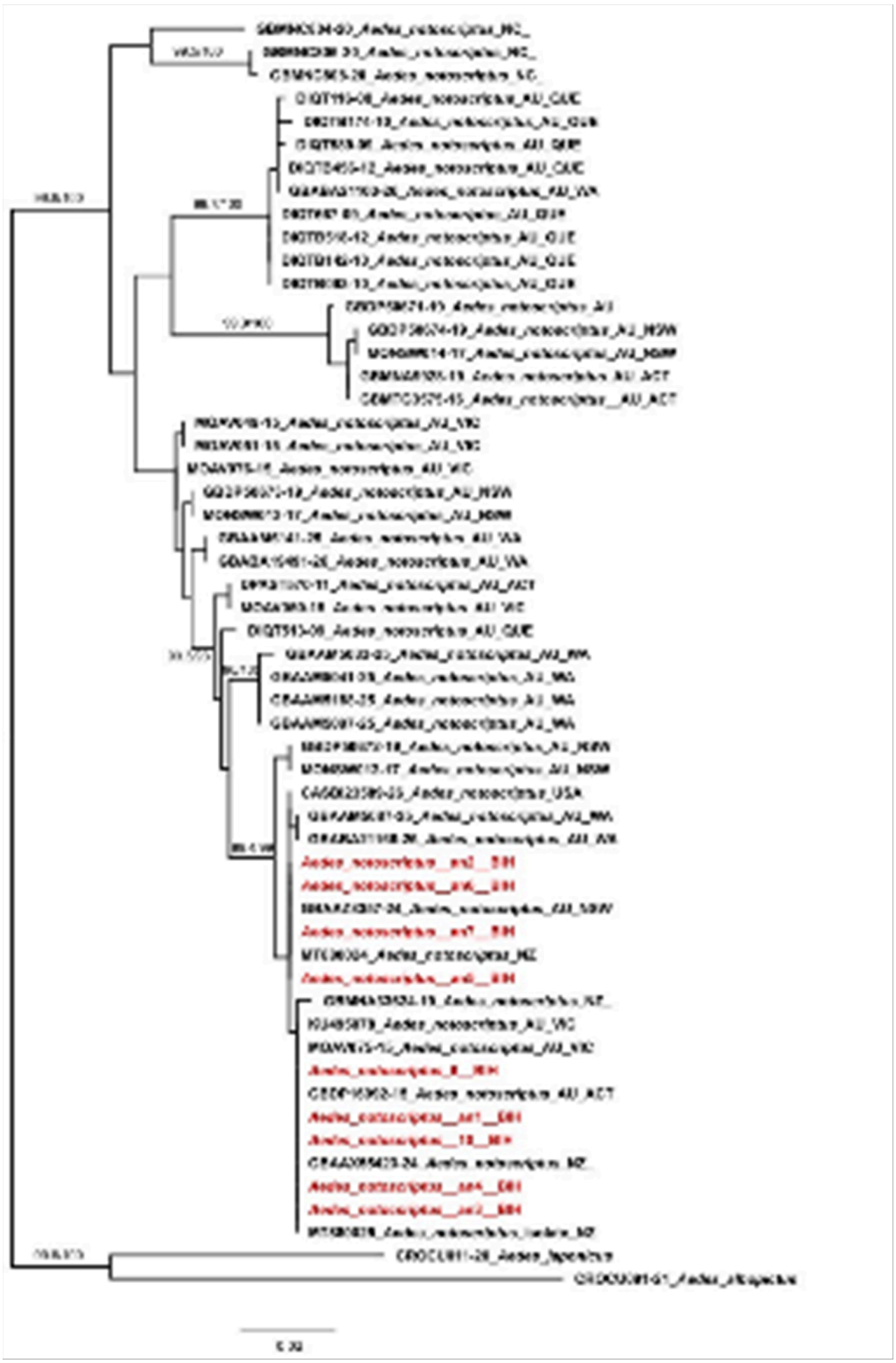


Figure 4

Phylogenetic tree of *Ae. notoscriptus* obtained using the maximum likelihood (ML) method. BiH specimens are shown in red font. Values next to nodes represent SH-aLRT/UFBoot support, with only

values ≥ 90% shown for clarity. The geographic origin of the analyzed samples (AU_ACT – Australia, Australian Capital Territory; AU_NSW – Australia, New South Wales; AU_QUE – Australia, Queensland; AU_VIC - Australia, Victoria; AU_WA - Australia, Western Australia; BIH - Bosnia and Herzegovina, Herzegovina; NC - New Caledonia; NZ - New Zealand).

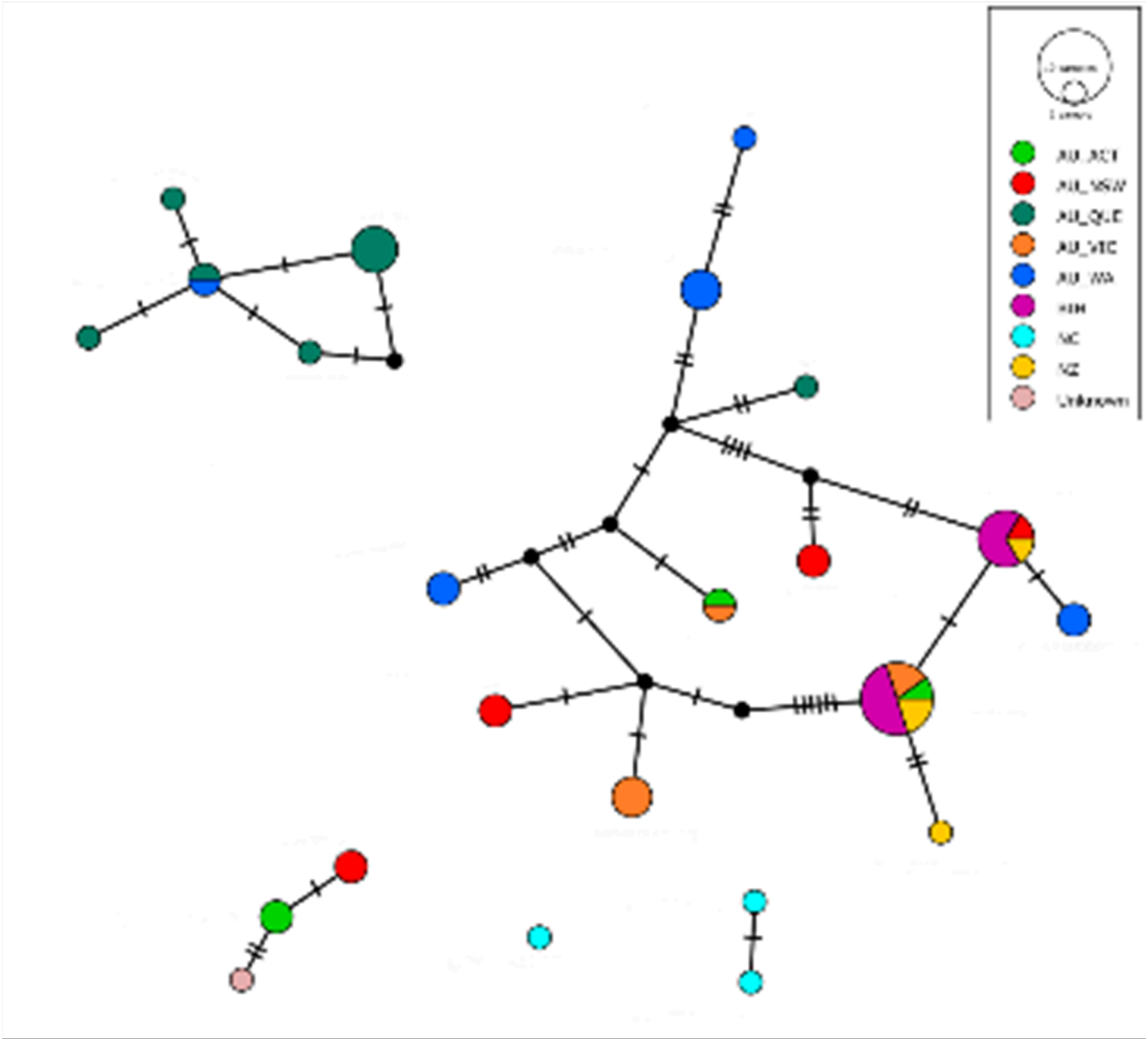


Figure 5

TCS haplotype network based on COI sequences of *Ae. notoscriptus* obtained in this study and reference sequences from publicly available databases (BOLD and GenBank). The area of each circle reflects the frequency of the corresponding haplotype, while colors indicate the geographic origin of the analyzed samples (AU_ACT – Australia, Australian Capital Territory; AU_NSW – Australia, New South Wales;

AU_QUE – Australia, Queensland; AU_VIC - Australia, Victoria; AU_WA - Australia, Western Australia; BIH - Bosnia and Herzegovina, Herzegovina; NC - New Caledonia; NZ - New Zealand). Lines connecting haplotypes represent single mutational changes, and black dots correspond to inferred intermediate (missing) haplotypes.

Supplementary Files

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- Additionalfile1TableS1.xlsx
- Additionalfile2Table2.xlsx
- GraphicalAbstract.tiff
- Additionalfile3TableS3.xlsx