



## First record of a medically important vector, the Asian tiger mosquito *Aedes albopictus* (Skuse, 1895) (Diptera: Culicidae), using morphological and molecular data in northern Iran

**Shahyad Azari-Hamidian**

Research Center of Health and Environment, School of Health, Guilan University of Medical Sciences, Rasht, Iran [1]; Department of Medical Parasitology, Mycology and Entomology, School of Medicine, Guilan University of Medical Sciences, Rasht, Iran [2].

✉ [azari@gums.ac.ir](mailto:azari@gums.ac.ir)

<https://orcid.org/0000-0002-9370-9638>

**Behzad Norouzi, Hannaneh Maleki**

Research Center of Health and Environment, School of Health, Guilan University of Medical Sciences, Rasht, Iran.

✉ [behzadnorouzi66@yahoo.com](mailto:behzadnorouzi66@yahoo.com)

<https://orcid.org/0000-0002-9788-5890>

✉ [maleki.hana78@gmail.com](mailto:maleki.hana78@gmail.com)

<https://orcid.org/0000-0001-6952-7319>

**Seyed Mahmoud Rezvani, Morteza Pourgholami**

Vice-Chancellorship of Health, Guilan University of Medical Sciences, Rasht, Iran.

✉ [s.m\\_rezvani@yahoo.com](mailto:s.m_rezvani@yahoo.com)

<https://orcid.org/0009-0006-2092-1909>

✉ [morteza\\_66@yahoo.com](mailto:morteza_66@yahoo.com)

<https://orcid.org/0000-0002-1921-4784>

**Mohammad Ali Oshaghi**

Department of Vector Biology and Control of Diseases, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran.

✉ [moshaghi@tums.ac.ir](mailto:moshaghi@tums.ac.ir)

<https://orcid.org/0000-0003-3004-0364>

**ABSTRACT.** Iranian mosquitoes (Diptera: Culicidae) include 73 species across eight genera. The fauna of mosquitoes in Guilan Province comprises 34 species classified into seven genera. A faunistic study of mosquitoes was conducted with emphasis on an aggressive biter mosquito reported by local people in Anzali and Rasht Cities of Guilan Province, northern Iran. Collections were made by hand catches using manual aspirators, light traps, BG lure traps and ovitraps during August–November 2023 in all 17 counties of the province. Species identification was carried out using morphological keys and molecular analysis of the barcode region of the cytochrome c oxidase subunit I (COI) gene. In total, 29 larvae and 896 adult specimens were collected which were not recognized as a species previously known to occur in the province. The aggressive biter mosquito was morphologically identified as *Aedes albopictus* (Skuse, 1895). The sequences of the barcode region of the COI gene of the species were generated for the first time in the country. This species was collected in 14 counties of the province. Thus, the mosquito fauna of Guilan Province increased to 35 species.

**Keywords:** Cytochrome c oxidase I, dengue fever, Guilan Province, invasive species, *Stegomyia albopicta*

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## INTRODUCTION

The biting nuisance and their role in the transmission of different pathogens make mosquitoes (Diptera: Culicidae) the most important arthropods in public health entomology (Wilkerson et al., 2021). The family includes two subfamilies, Anophelinae and Culicinae, 41 genera and a total of 3,726 species

**Corresponding author:** Azari-Hamidian, S., ✉ [azari@gums.ac.ir](mailto:azari@gums.ac.ir)

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(Harbach, 2024). Six known invasive *Aedes* species including *A. aegypti* (Linnaeus, 1762), *A. albopictus* (Skuse, 1895), *A. atropalpus* (Coquillett, 1902), *A. japonicus* (Theobald, 1901), *A. koreicus* (Edwards, 1917) and *A. notoscriptus* (Skuse, 1889) (Azari-Hamidian, 2023). To date, Iranian mosquitoes consist of 73 species representing eight genera (Azari-Hamidian et al., 2019, 2024). The fauna of mosquitoes in Guilan Province, northern Iran, includes 34 species representing seven genera (Azari-Hamidian & Norouzi, 2018; Azari-Hamidian et al., 2024).

*Aedes albopictus* is cosmopolitan, although originally confined to the Oriental Region, and occurs in Armenia, Oman, Pakistan and Türkiye neighbouring Iran (Azari-Hamidian et al., 2019; Wilkerson et al., 2021). This species is a known aggressive biter and invasive mosquito and is of great medical and veterinary importance as a competent vector of at least 22 arboviruses including chikungunya, dengue, West Nile and Zika viruses, all of which, except for Zika virus, have been recorded in Iran (Gratz, 2004; Azari-Hamidian & Harbach, 2023). Also, it is a known vector of some avian plasmodia such as *Plasmodium fallax*, *P. galliceum* and *P. lophurae* and filarial nematodes such as the dog heart worm *Dirofilaria immitis* and *D. repens* (Gratz, 2004; Wilkerson et al., 2021). There is one record of *A. albopictus* in Sistan and Baluchistan Province of southeastern Iran (Doosti et al., 2016). However, after that, this species has not been collected in the province and other areas of southern Iran in other surveys (Keshavarzi et al., 2017; Soltani et al., 2017; Nejati et al., 2020, 2024; Jaberhashemi et al., 2022). Thus, it seems that the species has not been established in the region.

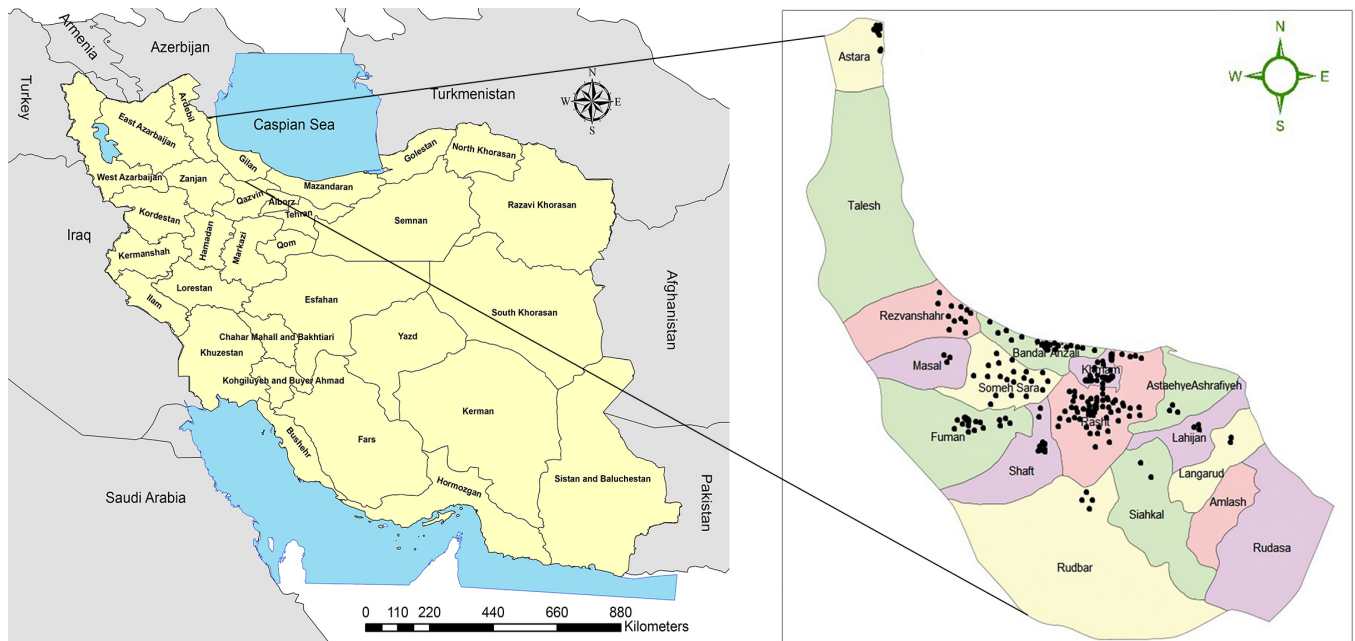
Consistency between morphological identification, as the gold standard, and identification using the barcode region of the cytochrome c oxidase subunit I (*COI*) gene were reported from 69% to 98% and the *COI* barcode region can be successfully used to identify mosquito species (Ruiz-Lopez et al., 2012; Talaga et al., 2017; Laurito et al., 2022; Wu et al., 2022). Inconsistency between morphological identification and barcoding might be due to misidentification, synonymy, evidence of species complex, some degree of hybridization, incomplete lineage sorting between species and evidence of the effect of the geographical scale of sampling (Laurito et al., 2022). Although there are a few reports on the *COI* barcode region for some species of the genera *Aedes*, *Anopheles* and *Culex* in Iran (Azari-Hamidian et al., 2009, 2010; Karimian et al., 2014; Koosha et al., 2017; Nejati et al., 2024); however, there is no information about the *COI* barcode region for many other mosquito species of the country.

The aim of this study was a faunistic investigation of mosquitoes using morphological and molecular data with emphasis on an aggressive biter mosquito reported by local people in Anzali and Rasht Cities of Guilan Province.

## MATERIAL AND METHODS

**Sampling and morphological identification.** Initial hand catch collections were made by manual aspirators in the reported localities, Anzali and Rasht Cities, during August 2023, followed by extensive collections using manual aspirators, light traps, BG lure traps and ovitraps up to November 2023 in all 17 counties of Guilan Province (Fig. 1). The specimens were transported to the Laboratory of Medical and Veterinary Entomology of the School of Health, Guilan University of Medical Sciences. Species identification was carried out utilizing morphological identification keys (Huang, 2004; Azari-Hamidian & Harbach, 2009; Becker et al., 2020). Adult voucher specimens are deposited in the Museum of Medical and Veterinary Entomology, the School of Health, Guilan University of Medical Sciences.

**DNA barcoding.** Molecular analysis was carried out using the sequences of the barcode region of the *COI* gene of mitochondrial DNA (mtDNA). The polymerase chain reaction (PCR) mixes and thermocycler parameters were those described by Folmer et al. (1994). The barcode region of the *COI* gene was amplified using the LCO (5'-GGT CAA CAA ATC ATA AAG ATA TTGG-3') and HCO (5' -TTA AAC TTC AGG GTG ACC AAA AAA TCA-3') primers (Folmer et al. 1994). The product was 709 bp (658 bp without primers) for all mosquitoes. The sequences were generated in both directions and edited and aligned using MEGA v. 7 (Kumar et al., 2016).



**Figure 1.** Map of Iran highlighting the location of Guilan Province in northern Iran where the specimens of *Aedes albopictus* (Skuse, 1895) were collected in 2023.

Pairwise sequence divergence and the maximum likelihood tree, using 1,000 replicates, based on the Kimura's two-parameter (K2P) model were obtained using MEGA v. 7 (Kimura, 1980; Kumar et al., 2016). Similarity with sequences in GenBank was assessed using BLAST (<http://blast.ncbi.nlm.nih.gov>). The sequences which were generated with the aforementioned primers and 100% coverage were chosen from GenBank for comparison. Template DNA from this study is retained in the Molecular Laboratory of the School of Health, Guilan University of Medical Sciences.

## RESULTS

### *Taxonomic hierarchy*

**Class Insecta Linnaeus, 1785**

**Order Diptera Linnaeus, 1758**

**Suborder Nematocera Duméril, 1805**

**Infraorder Culicomorpha Hennig, 1948**

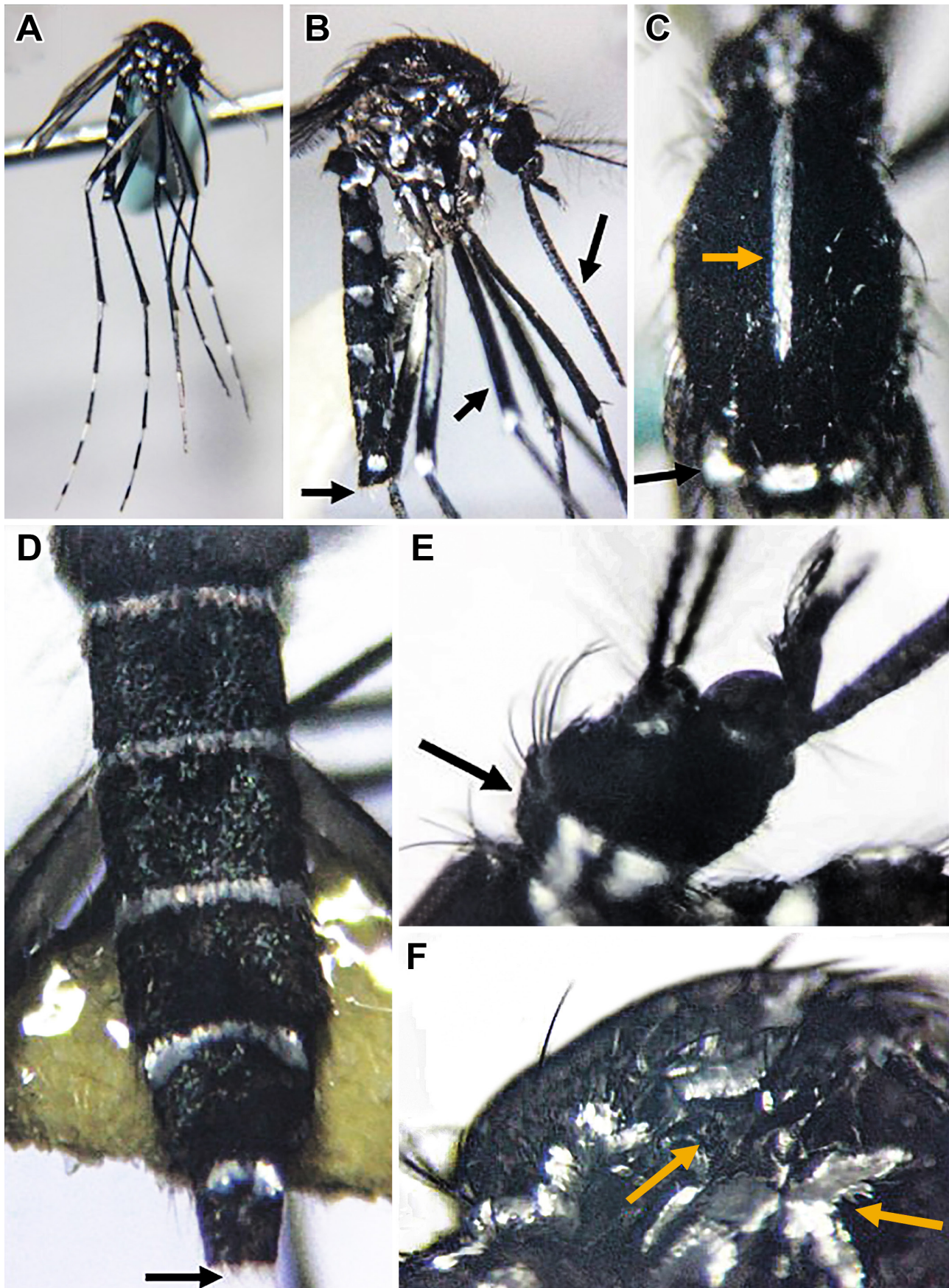
**Family Culicidae Meigen, 1818**

**Genus *Aedes* Meigen, 1818**

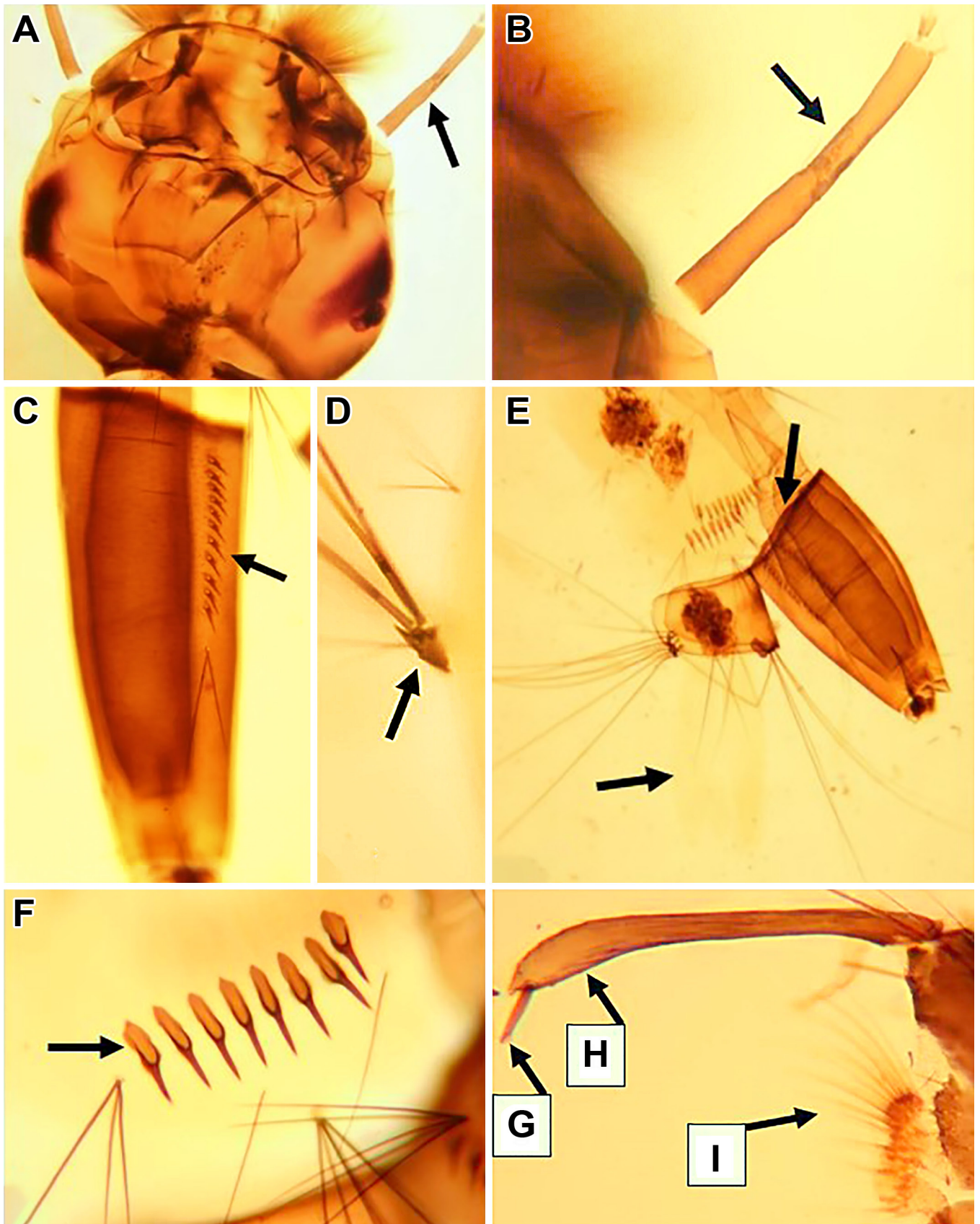
**Subgenus *Stegomyia* Theobald, 1901**

***Aedes albopictus* (Skuse, 1895)**

**Material examined.** In total, 29 larvae, were reared from the eggs, and 1529 adult specimens were collected. Among them, 29 larvae and 896 adult specimens were caught which were not recognized as a species previously known to occur in the province. They were captured using manual aspirators (886 specimens), BG lure traps (10 specimens) and ovitraps during August–November 2023. The aggressive biter mosquito species was morphologically identified as *A. albopictus* (Figs 2 and 3). This species was collected in 14 counties of the province, out of 17 (Fig. 1).



**Figure 2.** Female of *Aedes albopictus* (Skuse, 1895), captured in Guilan Province, northern Iran in 2023. **A.** General habitus; **B.** Proboscis; **C.** Thorax, dorsal view; **D.** Abdomen, dorsal view; **E.** Head; **F.** Thorax, lateral view.



**Figure 3.** Larva and male genitalia of *Aedes albopictus* (Skuse, 1895), captured in Guilan Province, northern Iran in 2023. **A.** Head and antenna; **B.** Seta 1-A; **C.** Pecten; **D.** basal tubercles of 9-12-M; **E.** Annal papillae and auricle (absent); **F.** Comb; **G.** Spine of the gonostylus; **H.** Gonostylus; **I.** Mesal surface of the gonocoxite.

Other species, which were collected with *A. albopictus* by hand catches, were: *A. caspius* (Pallas, 1771) s.l. (4 females), *A. cinereus* Meigen, 1818 (94 females), *A. vexans* (Meigen, 1830) (312 females), *Coquillettidia richiardii* (Ficalbi, 1889) (7 females), *Culex pipiens* Linnaeus, 1758 (116 females), *C. tritaeniorhynchus* Giles, 1901 (82 females) and *Uranotaenia unguiculata* Edwards, 1913 (2 females). Also, *C. pipiens* (12 females) and *C. tritaeniorhynchus* (4 females) were collected with *A. albopictus* using BG lure traps. Also, 29 larvae were reared from the eggs, collected by egg traps, and were morphologically identified as *A. albopictus* (Fig. 3). Moreover, four microscope slides of the male genitalia verified the morphological identification of females and larvae (Fig. 3). No specimen of the species was captured by means of light traps.

**DNA barcoding.** The sequences of the *COI* barcode region were obtained from two specimens of *A. albopictus* collected from Ghalamgoudeh, Anzali, 37°27'39.6"N, 49°27'43.2"E, 6.VIII.2023 (GenBank accession number: PP564390) and Astará City, 38°25'26.4"N, 48°52'12"E, 19.X.2023 (GenBank accession number: PP564391). The nucleotide sequences were 709 bp (658 bp without primers) as expected. The *COI* sequences of specimens from Anzali and Astará shared 100% coverage and identity with each other, one haplotype, and identical with the sequences in GenBank of specimens of *A. albopictus* from China (Shanghai, KX266726.1), Italy (JX679374.1), South Korea (MW829500) and Türkiye (MK714006.1). Eight selected sequences with 100% coverage were compared from China (MT890465.1, MZ007511.1, OR237214.1), Congo (MN299017.1), Germany (JQ388786.1), India (KJ410335.1), Malaysia (KY817524.1) and Thailand (OK413073.1) (similarity range 99.54–99.85%) (Fig. 4, Table 1). Variable bases of the *COI* barcode sequences obtained from the Iranian specimens and overlapping sequences from GenBank were presented in Table 1. Translation of nucleotide sequence to amino acids resulted in an alignment of 219 amino acids, eight (3.6%) of which were variable (Table 1).



**Figure 4.** Consensus tree of cytochrome c oxidase I (*COI*) barcode sequences of *Aedes albopictus* (Skuse, 1895) collected in Anzali and Astará Cities, Guilan Province of northern Iran, 2023, using maximum likelihood method based on the Kimura's two-parameter (K2P) model.

**Table 1.** Variable bases of the cytochrome c oxidase I (*COI*) barcode sequences generated from the specimens of *Aedes albopictus* (Skuse, 1895) found in Anzali and Astara Cities, Guilan Province of northern Iran, 2023, with overlapping sequences from GenBank. Dots stand for identical bases as the Iranian specimens.

GenBank Accession Numbers	Nucleotide Position							
	31	49	62	346	373	553	589	628
PP564390, Iran, Anzali	T	A	A	T	G	C	C	G
PP564391, Iran, Astara	.	.	.	.	.	.	.	.
MW829500.1, South Korea	.	.	.	.	.	.	.	.
MK714006.1, Türkiye	.	.	.	.	.	.	.	.
KX266726.1, China: Shanghai	.	.	.	.	.	.	.	.
JX679374.1, Italy	.	.	.	.	.	.	.	.
JQ388786.1, Germany	.	.	G	.	.	.	.	.
KJ410335.1, India	.	.	.	C	.	.	.	.
MT890465.1, China	.	.	.	C	.	.	.	.
OR237214.1, China	.	.	.	.	A	.	.	A
OK413073.1, Thailand	.	.	.	C	.	.	T	.
MZ007511.1, China	.	.	.	C	.	.	.	A
MN299017.1, Congo	.	.	.	C	.	.	.	A
KY817524.1, Malaysia	C	G	.	.	.	T	.	.

## DISCUSSION

Based on the present record, the occurrence of *A. albopictus* in Iran is verified and it is found for the first time in Guilan Province, adding this invasive species to the mosquito fauna of the province. Henceforth, the current mosquito fauna of Guilan Province includes 35 species. Also, the sequences of the *COI* barcode region were obtained from the specimens of this species for the first time in the country. In the previous report of this species in southeastern Iran, different parts of the *COI* region (including 460 base pairs) were amplified using different primers (Doosti et al., 2016; GenBank accession number: KU351083). Thus, it was not possible to compare the sequences generated in the present work with the previous sequence from Iran. In addition to the nucleotide sequences from Asia and Europe which displayed 100% coverage and similarity to the present investigation sequences (Fig. 4, Table 1), there are numerous sequences from Africa, Asia and Europe with 100% coverage and more than 99% similarity in GenBank. Eight selected sequences were used to compare (Fig. 4, Table 1). All those selected sequences displayed less than one percent difference in nucleotides (range 0.15–0.46%). Thus, the variation of sequences generated in the present investigation surely is within intra-specific variation (Ruiz-Lopez et al., 2012; Hernández-Triana et al., 2019; Madeira et al., 2021; Chaiphongpachara et al., 2022; Wu et al., 2022).

In view of the recent finding of the Asian tiger mosquito in northern Iran, it is necessary to engage the national surveillance program for monitoring its population and interventions for its control. Studies of the bionomics, host preferences and resistance situation of the species to different insecticides in the region are recommended.

## AUTHOR'S CONTRIBUTION

The authors confirm their contribution to the paper as follows: S. Azari-Hamidian: conceptualization, methodology, investigation, data curation, writing original draft, resources, supervision; B. Norouzi: investigation, data curation, writing original draft; H. Maleki: investigation, data curation, writing original draft; S.M. Rezvani: investigation, data curation, writing original draft, resources; M. Pourgholami: investigation, data curation, writing original draft; M.A. Oshaghi: methodology, investigation, data curation, writing original draft. All authors have read and agreed to publish this manuscript.

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## AVAILABILITY OF DATA AND MATERIAL

Sequence data that support the findings of this study have been deposited in the GenBank (GenBank accession numbers: PP564390, PP564391). The specimens mentioned in this article are deposited in the Museum of Medical and Veterinary Entomology, the School of Health, Guilan University of Medical Sciences. Template DNA from this study is retained in the Molecular Laboratory of the School of Health, Guilan University of Medical Sciences. All other data are provided within the manuscript.

## ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study only included arthropod material, and all required ethical guidelines for the treatment and use of animals were strictly adhered to in accordance with international, national, and institutional regulations. No human participants were involved in any studies conducted by the authors for this article.

## CONSENT FOR PUBLICATION

Not applicable.

## CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this paper.

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## نخستین گزارش ناقل مهم پزشکی، پشه ببر آسیایی (*Aedes albopictus* (Skuse, 1895) (Diptera: Culicidae)، با استفاده از داده‌های ریخت‌شناختی و مولکولی در شمال ایران

شهید آذری حمیدیان<sup>۱\*</sup>، بهزاد نوروزی<sup>۱</sup>، حنا ملکی<sup>۱</sup>، سید محمود رضوانی<sup>۲</sup>، مرتضی پورغلامی<sup>۳</sup>، محمد علی عشاقی<sup>۴</sup>

۱ مرکز تحقیقات بهداشت و محیط زیست، دانشکده بهداشت، دانشگاه علوم پزشکی گیلان، رشت، ایران  
 ۲ گروه انگل‌شناسی، فارچ‌شناسی و حشره‌شناسی پزشکی، دانشکده پزشکی، دانشگاه علوم پزشکی گیلان، رشت، ایران  
 ۳ معاونت بهداشتی، دانشگاه علوم پزشکی گیلان، رشت، ایران  
 ۴ گروه بیولوژی و کنترل ناقلین بیماری‌ها، دانشکده بهداشت، دانشگاه علوم پزشکی تهران، تهران، ایران

\* پست الکترونیک نویسنده مسئول مکاتبه: [azari@gums.ac.ir](mailto:azari@gums.ac.ir)

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**چکیده:** پشه‌های ایران (Diptera: Culicidae) شامل ۷۳ گونه در هشت جنس است. فون پشه‌های استان گیلان شامل ۳۴ گونه در هفت جنس است. مطالعه فونستیک پشه‌ها با تاکید بر یک گونه به شدت خونخوار گزارش شده توسط مردم محلی در شهرهای انزلی و رشت در استان گیلان انجام شد. نمونه‌گیری با استفاده از روش دستی با ابزار آسپیراتور، تله‌های نورانی، تله بی جی لور و تله جمع‌آوری تخم در تیرماه تا آبان ماه سال ۱۴۰۲ در تمام ۱۷ شهرستان استان گیلان انجام شد. تعیین هویت گونه‌ها با استفاده از کلیدهای شناسایی ریخت‌شناختی و تحلیل مولکولی قطعه بارکد ژن *COI* انجام شد. در مجموع ۲۹ لارو و ۸۹۶ نمونه پشه جمع‌آوری شده به عنوان گونه‌ای مشخص گردید که پیش از این از این استان گزارش نشده بود. گونه به شدت خونخوار به صورت ریخت‌شناختی *Aedes albopictus* (Skuse, 1895) تشخیص داده شد. قطعه بارکد ژن *COI* برای این گونه برای نخستین بار در کشور فراهم گردید. این گونه از ۱۴ شهرستان استان جمع‌آوری شد. به این ترتیب فون پشه‌های استان گیلان به ۳۵ افزایش یافت.

**واژگان کلیدی:** ژن *COI*، تب‌دنگی، استان گیلان، گونه مهاجم، *Stegomyia albopicta*