

Original Article

Composition of *Anopheles* Species Collected from Selected Malarious Areas of Afghanistan and Iran

Helen Hoosh-Deghati ^{1,2}, *Navid Dinparast-Djadid ², *Vahideh Moin-Vaziri ¹, Hoda Atta ³, Abbas Ali Raz ², Seyyed Javad Seyyed-Tabaei ¹, Naseh Maleki-Ravasan ², Hamzeh Alipour ², Sedigheh Zakeri ², Eznollah Azar-Gashb ¹

¹Department of Parasitology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

²Malaria and Vector Research Group, Biotechnology Research Center, Pasteur Institute of Iran, Tehran, Iran

³Malaria Control, World Health Organization/Eastern Mediterranean Regional Office, Cairo, Egypt

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Abstract

Background: Malarious areas in Iran are close to Afghanistan and Pakistan that urge the researchers to extend their knowledge on malaria epidemiology to the neighboring countries as well. Vectorial capacity differs at species or even at population level, the first essential step is accurate identification of vectors. This study aimed to identify *Anopheles* species composition in selected malarious areas of Afghanistan and Iran, providing further applied data for other research in two countries.

Methods: Adults *Anopheles* spp. were collected from four provinces in Afghanistan (Badakhshan, Herat, Kunduz, Nangarhar) by pyrethrum spray catch, hand collection methods through WHO/EMRO coordination and from Chabahar County in Iran by pyrethrum spray catch method. Identification was performed using reliable identification key.

Results: Totally, 800 female *Anopheles* mosquitoes, 400 from each country were identified at species level. *Anopheles* composition in Afghanistan was *An. superpictus*, *An. stephensi* and *An. hyrcanus*. Most prevalent species in Badakhshan and Kunduz were *An. superpictus*, whereas *An. stephensi* and *An. hyrcanus* were respectively found in Nangarhar and Herat. *Anopheles* species in Chabahar County of Iran were *An. stephensi*, *An. fluviatilis*, *An. culicifacies* and *An. sergentii*. The most prevalent species was *An. stephensi*.

Conclusion: Current study provides a basis for future research such as detection of *Plasmodium* infection in collected samples which is on process by the authors, also for effective implementation of evidence-based malaria vector intervention strategies.

Keywords: *Anopheles*, Morphological identification, Afghanistan, Iran

Introduction

Malaria is known as one of the six important infectious diseases based on WHO (1). It is caused by the protozoon *Plasmodium* spp and transmitted by female *Anopheles* mosquitoes (2). *Anopheles* spp belongs to Culicidae family (Diptera) with 460 or so different species that 70 of them have the potential of being malarial vectors, however, around 36 species are considered as main ones (3, 4, 5). About 198 million cases of malaria occurred in 2013, leading to 584000 deaths (6). Glob-

ally, around 3.2 billion people are at risk of being infected with malaria and 1 in 1000 is at high risk of getting malaria in year (1). In Eastern Mediterranean regions (EMR), more than 10 million clinical malaria cases occur annually with 50 thousands death. Generally 287 million which is approximately 60% of Eastern Mediterranean population is at the risk of malaria infection (1, 7, 8).

Malaria is of great importance from public health concerns in Iran, more than 2 million

*Corresponding authors: Dr Navid Dinparast-Djadid, E-mail: navidmvr@gmail.com, Dr Vahideh Moin-Vaziri, E-mail: vmvaziri@gmail.com

people live at high risk regions (7). Iran with population of more than 78 million people is located in West Asia sharing a border of 1458 and 936km respectively with Iraq from the west, Afghanistan, and Pakistan from the east (9). Malaria is endemic in the South and Southeast of Iran, where more than 90 percent of malaria cases are reported (10, 12). These regions are bordered with Pakistan and Afghanistan which malaria cases due to *P. falciparum* and *P. vivax* are prevalent. Malaria has an unstable pattern varying between 42% up 60% of total malaria cases with two seasonal peaks mostly in spring and autumn after the rainy seasons in the provinces located in South-East of Iran comprising Sistan and Baluchistan, Hormozgan and the tropical part of Kerman (13). Recently a notable reduction has been observed in malaria cases due to the report of Ministry of Health and Medical Education of Iran, which has led WHO to categorize Iran in the elimination phase (14). Nationwide campaign malaria elimination was launched by Iranian Government in 2010 in epidemic regions with the goal of becoming malaria-free country by 2025 (12, 14).

Vector control is the most efficient way to decrease the malaria transmission at the community level. Regarding to the recent Iranian mosquitoes checklist, seven different genera, 64 species and 3 subspecies are reported (15). Seven species of the genus *Anopheles* including *An. culicifacies* Giles s.l., *An. dthali* Patton, *An. fluviatilis* James s.l., *An. maculipennis* Meigen s.l., *An. sacharovi* Favre, *An. stephensi* Liston, and *An. superpictus* Grassi are identified as malarial vectors in Iran. Six species are known as its vectors in southeast of Iran: *An. culicifacies*, *An. stephensi*, *An. dthali*, *An. fluviatilis*, *An. pulcherrimus*, and *An. superpictus*. Among them, *An. stephensi* is the main vector of malaria in the area and considerably prefers human blood (16, 13, 12).

In Afghanistan, approximately half of the population is at the risk of malaria. There are at least six malaria vectors in Afghanistan:

Anopheles superpictus, *An. culicifacies*, *An. hyrcanus*, *An. pulcherrimus*, *An. fluviatilis* and *An. stephensi* (17).

Full understanding of malaria vectors can be considered as a key factor in malaria control programmes. The first step in any control program is the vector species identification. Moreover, malaria endemic areas in Iran are close to Afghanistan and Pakistan, urging us to extend our research to the neighboring countries as well.

Anopheles species were collected from selected malarious areas of Afghanistan (Badakhshan, Herat, Kunduz, and Nangarhar) located in high risk areas (18, 19) by WHO.

This study aimed to identify *Anopheles* species composition in selected malarious areas of Afghanistan and Iran, providing further applied data for other research in two countries. Results presented here reflect the composition of *Anopheles* species of mentioned collection as a part of ongoing project. It could provide also useful information in planning and implementing of an effective program for vector control during elimination phase in Iran and control phase in Afghanistan for the national malaria control program.

Materials and Methods

Study areas

Afghanistan: Afghanistan is a landlocked country in south central Asia (33° 00' N, 65° 00' E). Its climate is arid to semiarid with frequent sand storms. The northern and southeastern areas of the country have very rugged mountain terrains (Hindu Kush), while the western and southern parts are flat deserts and plains. Climate varies according to the elevation and location. In general, malaria occurs at altitude below 2,000m and is the most prevalent in snow-fed river valleys and rice growing areas (20). Samples were collected from four counties, located in different geographical sites of Afghanistan, North-East (Badakhshan and Kunduz), South-East (Nangarhar) and

Western part close to Iran boundary (Herat) (Fig. 1).

Iran: Sistan and Baluchistan Province as the main malarious area is located in the Northern coast of Persian Gulf and Oman Sea, where the weather is warm and humid enough for *Anopheles* species to be activated throughout the year (7).

This study took place in Chabahar County (25° 17' N, 60° 38' E), bounded by Pakistan in the east, Hormozgan Province in the west, Kerman Province in the north, and Oman Sea in the south. It has a warm and humid weather in summer and temperate in winter. The average maximum and minimum temperature are respectively 34 °C and 10.5 °C in July and January. Latitude from the sea level is 7m. The annual rainfall is low mainly in spring and autumn so that it has a desert climate. However it has high humidity due to neighboring sea (Fig. 1) (21).

Mosquito collection and identification

Adult *Anopheles* spp were collected on August 2011 through WHO/EMRO coordination from four provinces of Afghanistan (Badakhshan, Herat, Kunduz, Nangarhar) by pyrethrum spray catch, hand collection method using manual aspirator and from Chabahar County in Iran by pyrethrum spray catch method (Table 1). The sampling sites are indicated on Fig. 1. The species identification was performed by using reliable identification key (22) under Leica EZ4D (16X) stereomicroscope.

Results

Morphological identification of Afghanistan Anophelinae mosquitoes

A total of 400 adults female *Anopheles* were collected from Herat, Nangarhar, Badakhshan and Kunduz provinces of Afghanistan (Table 1). Among the collected specimens, 3 species were identified based on the morphological characteristics, 45% of total specimens belong to *An. superpictus* collected from Badakhshan and

Kunduz. Totally 180 *An. superpictus* were collected from mentioned areas, with pyrethrum spray catch (PSC) and hand collection, among them 141 (78.3%) collected from human house. The major morphological characteristics of this species are having unusually long palps (Fig. 2A), no dark spots at point of bifurcation of 5th longitudinal vein and equal division part of the second and fourth vein to the end of wing (Fig. 2B). Next species was *An. hyrcanus* which was gathered by PSC method from animal and human house in Herat. Totally 110 (27.5%) was identified, which mainly collected from human house (63%). In *An. hyrcanus* fore margin of wing has less than four light and four dark bands alternating (Fig. 3A), fore margin of the wing with two small white spots, one near the tip, the other about two thirds of the way from the root of the wing (Fig. 3B).

Third identified species was *An. stephensi* (27.5%), which was sampled from the human house of Nangarhar by PSC method. This species could be easily characterized by white spots which speckled on femora and tibiae (Fig. 4 A, B).

Morphological identification of Anopheline mosquitoes of Chabahar County (Iran)

Morphological characteristic of 400 adults female *Anopheles* specimens collected from two villages of Chabahar County, Iran, revealed the presence of 4 different species (Table 1). Regardless the kind of species, all specimens were collected from human house and by PSC method. The most prevalent species among them was *An. stephensi* (94.75%), which its main morphology character was mentioned previously. The other species were *An. fluviatilis* (2.75%), *An. culicifacies* (1.5%) and *An. sergentii* (1%). *Anopheles culicifacies* has been identified based on the following main characters, existence of two fringe spots, no tuft of scales on anterior margin of mesonotum and finally existence of a dark spot opposite to the light spot at the costa

base (Fig. 5). *Anopheles fluviatilis* has palps with usual length (Fig. 6A), dark spot at the point of bifurcation of the 5th vein and farther division part of the second vein than the fourth vein with respect to the end of wing (Fig. 6B).

Anopheles sergentii characteristics include more than two light spots on the posterior margin of fringe with pale base of subcostal and no dark spot of sub-costal opposite to the pale spot of costa (Fig. 7).

Table 1. Details of collected specimens based on study areas and collection methods: PSC: Pyrethrum Spray Catch, HC: Hand Collection by manual aspirator, HC: Hand Collection, HH: Human House, AH: Animal House

Province	Village	Collection methods and Sites	Number of Mosquitoes	<i>Anopheles</i> spp	Total number of species	Total	
Afghanistan	Herat	PSC-HH (Indoor)	70	<i>An. hyrcanus</i>	110 (27.5%)	400	
		PSC-AH (Indoor)	30				
	Nangarhar	Pol-e tar-acheh	PSC-HH (Indoor)	55	<i>An. stephensi</i>		110 (27.5%)
		Ali khan		55			
	Badakhshan	Darewazer	PSC-AH (Indoor)	39	<i>An. superpic-tus</i>		100 (25%)
			HC-HH (Indoor)	37			
		PSC-HH (Indoor)	24				
Kunduz	Gerghiz	PSC-HH (Indoor)	80	<i>An. superpic-tus</i>	80 (20%)		
Iran	Chabahar	PSC-HH (Indoor)	100	<i>An. stephensi</i>	379 (94.75%)	400	
				<i>An. fluviatilis</i>	11 (2.75%)		
		PSC-HH (Indoor)	300	<i>An. culicifa-cies</i>	6 (1.5%)		
	Baloch-adam			<i>An. sergentii</i>	4 (1%)		



Fig. 1. Map of Iran and Afghanistan, indicating the location of the study area in Chabahar County (marked with an asterisk) situated in the south of Sistan and Baluchistan in Iran and Herat, Kunduz, Badakhshan, Nangarhar provinces in Afghanistan.



Fig. 2. *Anopheles superpictus*, A: Head with long palps B: No dark spot at point of bifurcation of 5th vein and division part of the second and fourth vein to the end of wing is equal

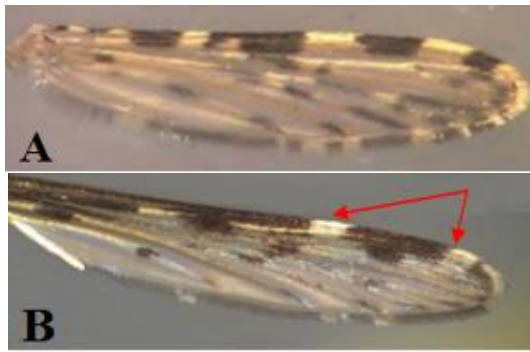


Fig. 3. A: existence at least dark spot at the upper margins of the wings, which is common in all *Anopheles* except *An. hyrcanus* B: *An. hyrcanus*, fore margin of the wing with two small white spots

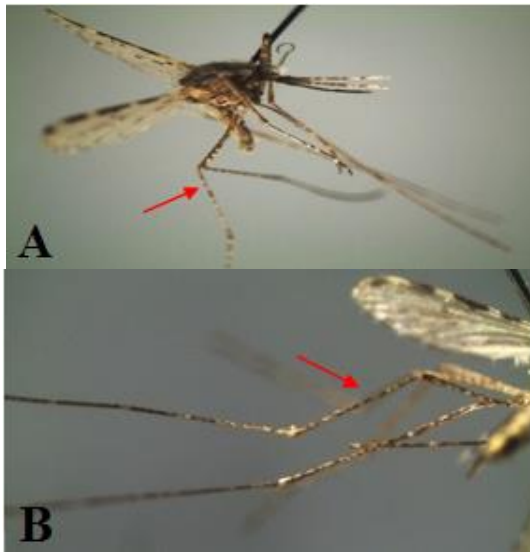


Fig. 4. *Anopheles stephensi*, A, B: Femora and tibiae speckled with white spots



Fig. 5. *Anopheles culicifacies*, first vein with a dark spot opposite the light spot at the base of costa



Fig. 6. *Anopheles fluviatilis*, A: usual length of palps B: spot at the point of bifurcation of the 5th vein and division part of the second vein is farther than the fourth vein to the end of wing

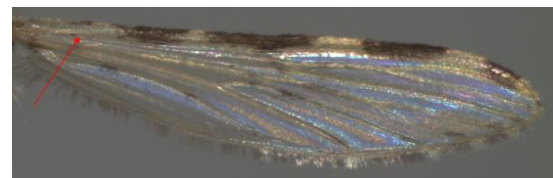


Fig. 7. *Anopheles sergentii*, no dark spot on sub-costa

Discussion

Malaria is widespread in the tropical and subtropical regions of the world and is also a public health problem in western extensions of the oriental zoogeographical region including Iran, Afghanistan and Pakistan. According to WHO report, 9 of the 97 countries with ongoing malaria transmission, including Iran, are classified as being in the malaria elimination phase. At this stage, interventions focus on the detecting all malaria cases, managing malaria foci, managing imported malaria cases and preventing onward transmission (23). Regarding the latest reports, it should be intentioned that, different *Anopheles* species even different pop-

ulation of one species, have different ecologies, biological attributes and vectorial capacities (24). Then, exact species identification is necessary for better understanding of their potential roles in malaria transmission (25, 12).

In Iran the most problematic malarious areas is south and southeast of the country, which highly affected by the epidemiology of malaria in neighboring countries, Afghanistan and Pakistan. The following reasons support this consumption 1. Afghanistan is in control phase of the malaria control, compared to Iran which is in elimination phase (14), 2. similarity of *Anopheles* fauna and human causative agents of malaria, both *P. falciparum* and *P. vivax* are prevalent in both countries and 3. importance of immigration from Afghanistan and Pakistan to malarious areas of Iran is the major routes of malaria transmission. This situation urges us to conduct current study, concentrating on morphological characters of *Anopheles* and their composition within selected areas of Afghanistan and Iran. Taxonomy that utilizes morphological characterization has been known as the gold standard method in identifying the mosquito species, which also have been used in current study for identification of five anopheline species in the study areas of Iran and Afghanistan, including known malaria vector species.

All the species which were identified during current study previously was reported in the checklist of Iranian mosquito fauna (15). The most prevalent species among our collected specimens from Chabahar was *An. stephensi* playing a dominant role in transmission of malaria in Persian Gulf as well in south of Iran (26, 27). This species is considered as an endophage and endophile mosquito. Sporozoite rate of this species was reported between 0.2 and 1.8% in south of Iran (26, 28) Other collected species were *An. culicifacies*, *An. fluviatilis* and *An. sergentii*; the first two types are also important as secondary vectors in the South and South-East of the country (3, 12).

Among secondary vectors, *An. culicifacies*

can be regarded as potential vector, as it is greatly responsible for the epidemic of malaria in Sistan and Baluchistan Province (29). Sporozoite rate of this species is varied between 1–4.7% in south malarious areas of Iran (3, 12). Although it is mainly known as zoophilic *Anopheles*, its anthropophilic index is reported as 16.4% (30). *Anopheles culicifacies* has also a wide distribution in Asia and Indian subcontinent (31). *Anopheles fluviatilis*, distribution at altitude 50–1100m, along the foothills of the Zagros Mountains and extension to South and South-East of Iran as a secondary vector, its sporozoite rate was reported between 1.4–11% (3, 12). *Anopheles sergentii* is also prevalent in different provinces of Iran, acting as secondary vector and responsible for maintaining the parasite reservoir in malarious areas.

In Afghanistan, totally three species were identified among collected specimens, reported previously in mosquito fauna of this country (32). Comprising *An. superpictus*, *An. stephensi* and *An. hyrcanus*, which are all known vectors of malaria (33). *Anopheles superpictus* has a broad geographical distribution in Asia, Europe and Africa considered as a malaria vector in these areas (13, 12). It was collected from Badakhshan and Kunduz located in northeast of Afghanistan as it was reported previously (34). Moreover, the only species which was recently reported by Ahmad et al. from the Badakhshan was *An. superpictus* (35). Although during current study, this species was not found among the collected specimens of Chabahar, but it is commonly widespread in Iran in altitudes 50–2000m. Sporozoite rate of mentioned species is 0.65–4.7% in Iran (36, 3). *Anopheles stephensi* was collected mainly from Nangarhar Province in human house and mainly were blood-fed. As mentioned, this species is an important vector of human malaria in the Middle East and South Asia including Indo-Pakistan sub-continent (16). The third species in the study area was *An. hyrcanus*. It is a mem

ber of the Hyrcanus group comprising of 30 species, some of them are vectors of malaria, filariasis and arboviruses (37, 38). It is considered as the fauna of bloodsucking mosquitoes of Afghanistan (32, 17), also its potential role as malaria vector was reported from France, Turkey, Greece and Afghanistan (32, 39). This species has already been reported in the North and North-East of Iran (40), and is suspected to transmit malaria in Fooman District located in the north of Iran (41).

This study presents some limitations that may reflect the article. Mosquito collection was performed in a limited time and areas of Iran and Afghanistan due to the shortage of research time and lack of facilities, so although results are worthy enough for the other basic research, but could not regarded as a representative of a faunistic or eco-epidemiological study.

Conclusions

Obtained results provide baseline information for other researchers and it reveals valuable information for designing further studies on vectorial capacity and detection of *Plasmodium* species and the status of insecticide resistance within collected *Anopheles* spp which is undergoing by the authors. Furthermore, it could be useful for designing, implementation and evaluation of local and regional evidence-based malaria control program.

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