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Vector abundance and species composition of *Anopheles mosquito*in Central Region and Central West Highlands, Viet Nam

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Abstract

Malaria is a major cause of morbidity and mortality in Viet Nam. In 2008, World Health Organization reported an estimated value of 243 million cases with a mortality of about 863 thousand in the world. Malaria transmission in the Central Region and Central West Highlands of Viet Nam is known to be holoendemic and perennial. High humidity (80%) and a high mean temperature of 25°C in this area favour the bionomics of the principal malaria vectors. Vector control is a major component of the Global Malaria Control Strategy and still remains the most generally effective measure to prevent malaria transmission. Successful application of vector control measures requires the understanding of the bionomics of Anopheles species responsible for malaria transmission, including correct and precise identification of the target species and its distribution.This study was conducted to provide information on the vector abundance and species composition of Anopheles mosquito at Quang Binh, Binh Đinh, Khanh Hoa, Ninh Thuan, Gia Lai, Dak Lake of Central Region and Central West Highlands of Viet Nam. A total of 18 Anopheles species were collected in these provinces, therein, the 2main vectors are *An. minimus* and *An. dirus*, and the 3 secondary vectors are *An. aconitus, An. jeyporiensis, An. maculatus*. *An. dirus* and *An. minimus* species are present in most of the studied communes in 6 provinces, *An. aconitus, An. jeyporiensis* and *An. maculatus*are present in Binh Dinh, Ninh Thuan and Gia Lai provinces, while*An. jeyporiensis*does not seem to be found in the other provinces.

Keywords: Anopheles, Ethiopia, infectivity rate, malaria, mosquitoes, diseases vectors.

Introduction

Mosquitoesare known to be a vector for some of the most troublesome human parasites, including malaria which is a widely spread and communicable disease, especially in tropical area [1]. Global prevalence rate of malaria was estimated at 214 million cases with 438,000 deaths in 2015 [2].Malaria is a blood infection caused by mosquito (*Anopheles spp.*) borne apicomplexan parasites of the genus *Plasmodium* [3-5]. Of five *Plasmodium* species known to infect humans, *P. falciparum* causes by far the greatest morbidity and mortality, with several hundred million cases of clinical malaria [3-4].

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There are about 400 species of *Anopheles mosquitoes* in the world, but only about 60 of them are capable of transmitting malaria (WRBU - Traditional Mosquito Classification, September 2013) [6]. In Viet Nam, in 1966, Stojanovich and Scott described 41 species of Anopheles [7]. After 1957, a basic investigation regarding mosquito malaria was conducted nationwide. Sincethis period, many studies on mosquito malaria have been published. In 2008, the National Institude of Malariology, Parasitology and Entomology published about the Anophelinae mosquito in Viet Nam, which included 62 Anopheles species[8].To date, 64 species of Anopheles and 15 species of malaria have been identified in Viet Nam.The 3 vectors of malaria are*An. dirus, An. minimus* and *An. Epiroticus* [9]. The Central Region and Central West Highlands of Viet Nam havemore than 70% of the population living in areas at risk of malaria. This is the most complex malaria

situation in Viet Nam: in these regions, nearly 50% of the population get infected by the disease each year; with*Plasmodium* accounting for 75% of the cases.

The diversity of Anopheles mosquito poses a real challenge to malaria control programs because different species tend to have different behaviors and feeding locations (indoor versus outdoor) [10]. For example, if predominant Anopheles species within the region tend to feed on human outdoor, then common preventive measures like insecticide-impregnated bed nets and indoor spraying will be useless to prevent malaria. Also, the zoophilic tendency is also important in the case of zoophilic-anthropophilic Anopheles since the presence of domestic animals near housing will only attract mosquitoes [11].Since there are few studies about Anopheles species in Viet Nam, the exploration and mapping of this species and its distribution are important.To the best of our knowledge, this will be the first study to identify the presence of malaria and its vectors within this region. The main objectives of this study are to identify the species composition of man-biting mosquitoes,and to determine the diversity and dominance of the mosquito species found in the study villages using ecological indices. This project could therefore be used as baseline data to fight againstmalariaparasite, mainly by designing strategies to control itsvectorsinViet Nam.

Materials and Methods

Study Sites and Mosquitoes Collection

Study area: The study was carried out in 2014-2017 for six provinces including Quang Binh, Binh Đinh, Khanh Hoa, Ninh Thuan, Gia Lai, and ĐacLak, which are located in the Central Region and Central West Highlands of Viet Nam. All provinces are at a high risk of dengue epidemics.

Quang Binh is a province in the upper middle region of Viet Nam. It has a pretty narrow and steep topography since mountains cover 85% of the total area. Quang Binh belongs to the monsoon-tropical zone. The climate is divided into 2seasons. Rainy season lasts from September to March next year. The annual average rainfall is 2000-2300mm. Heavy rains concentrate in September, October and November. Dry season lasts from April to August. Annual average degree is 24ºC-26ºC.

Binh Dinh province lies along the south central coast of Viet Nam and is featured with typical tropical monsoon climate with an average temperature of 27°C. Rainy season normally lasts from September to December and the dry season is from January to August.

Khanh Hoa Province is located along the coastline of South Central Viet Nam.The climate ofKhanh Hoa is highly tropical. With an average temperature of 26.5°C, the weather is warm all the time in the plain regions.Khanh Hoahas a monsoon period which lasts from September to December.

Ninh Thuan province is lying in the middle of Central Viet Nam. Ninh Thuan lies within the driest region with tropical monsoon climate. The province has no winter and there are 2seasons a year: rainy season from September to November and dry season from December to August. The annual average temperature is 27°C. Ninh Thuan's weather is quite extreme with hot and dry atmosphere all year round.

Gia Lai is a Northern Province of the Center Highlands. Due to the average elevation of 700-800meters above sea level, the climate in Gia Lai is cool with 2distinct seasons: the rain season and the dry season. The rain season is from May to October and the dry season is from November to April, the average temperature is around 22-25°C.

Dak Lak is in Central West Highlandsat 400-800meters above sea level.The Dak Lak's climate is temperate with an annual average temperature at 24°C. There is a difference of only 5°C between the hottest month and the coldest one. The dry season lasts from November to April. It is quite cold, windy, and dry. The rainy season lasts from May to October with a high rain amount.

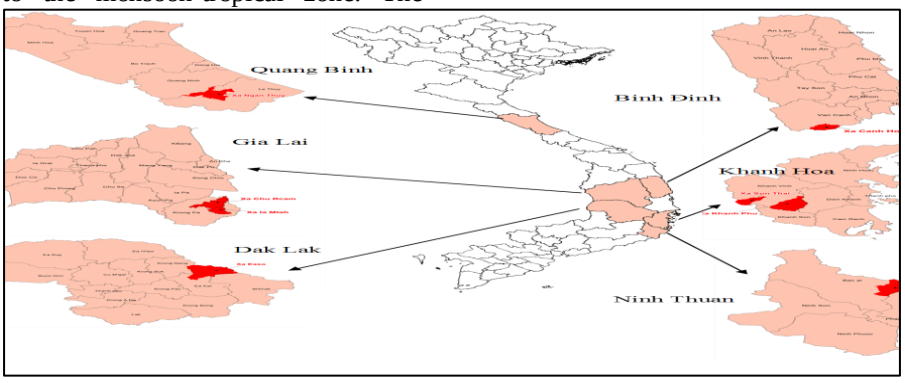

Fig1: Map of study sites

Mosquitoes analysis

Collection of the immature forms and rearing: The Anopheles mosquitoes larvae and pupae collection were conducted during the dengue outbreaks in 2014-2017 at all study sites. The field-collected larvae were stored in plastic tubes containing water from the same breeding habitat and brought to the insectary of Entomology Department where larvae were fed with larval food containing grounded dog biscuit, beef liver, powdered milk and yeast and reared to adults under standard conditions (25 ± 2°C temperature, 80 ± 10% relative humidity).

Anopheles mosquitoes and larvae morphologically were identified according to the Anopheles mosquito control index in Viet Nam (2008) of the National Institute of Malariology and Parasitology [6].

Mosquitoes collections

To establish species composition, collections were made inside the village, in the forest and on the way from the village to the forest. Outdoor and indoor human landing collections were made during eight nights per surveyby human landing-biting catches. Captures were performed by four individuals (2 groups of 2 people). The first group was seated 5-10m outside a house while the second group was seated inside a house. Collections were carried out every night from sunset to sunrise.At the end of each 1 h collection cycle, mosquitoes collected in vials were placed in plastic bags labeled with time and site of collection. The start of evening collections was set at 6 P.M.

CSP-ELISA

All collected specimens were subjected to ELISA to detect the circumsporozoite protein (CSP) of *P. falciparum*(P.f) with monoclonal antibody MAb P.f.2A10, P. vivax (P.v) 210 with monoclonal antibody Pv-210-CDC and P. vivax 247 with monoclonal antibody Pv-247-CDC in the head-thoracic portion of the mosquitoes. The captured monoclonal antibodies were bound to the plate, the well contents were aspirated and the remaining binding sites were blocked with blocking buffer (0.5% Casein technical, from bovine milk - Sigma, and 0.1N NaOH in PBS, pH 7.4). Mosquitoes to be tested were grounded in blocking buffer containing IGEPAL CA-630, and an aliquot was tested. Positive controls and negative controls (laboratory reared female Anopheles stephensi mosquitoes prepared in the same way as the test samples) were tested for each plate. After an incubation of 2hours at room temperature, the mosquito homogenate was aspirated and the wells were washed with PBS-Tween (0.05%). Peroxidase-linked monoclonal antibodies were then added to the wells. After 1 hour, the well contents were aspirated, washed again and the peroxidase substrate solution was added. After 30 minutes, the ELISA results were read by ELISA machine.

Results

Composition of Anopheles mosquitoes in study sites in Central Region and Central West Highlands in Viet Nam

We have collected Anopheles mosquitoes in august and november (rainy season) in Quang Binh, Binh Dinh, Ninh Thuan, and Dak Lak provinces, and in may and july (dry season), and inseptember and november (rainy season), in Gia Lai and Khanh Hoa provinces. Our results are shown in table 1.

Binh Dinh Province has collected 15 species of Anopheles, including the 2main vectors of malaria: *An. dirus* and *An.minimus*, and 3 other vector species: *An. aconitus, An. jeyporiensis* and *An. maculatus.* Of the 15 Anopheles species obtained, secondary vector *An. maculatus* is the one with the largest number of individuals.

Khanh Hoa province has collected 11 species of Anopheles, including one of the main malaria vector, An. Dirus, and 2secondary vector species: *An. aconitus* and *An. maculatus*. Of the 11 Anopheles species collected in Khanh Hoa, the 3 species of *An. dirus, An. maculatus, An. Peditaeniatus* have the highest number of individuals.

Ninh Thuan province has collected 14 species of Anopheles, including the 2 main vectors of malaria: *An. dirus* and *An.minimus*, and 3 secondary vector species: *An. aconitus, An. jeyporiensis* and *An. maculatus*. In the species collected in Ninh Thuan, the main vector *An. dirus* has the highest number of individuals.

Quang Binh province has collected 12 Anopheles species, including main vector of malaria *An.minimus*, and secondary vector species *An. aconitus* and *An. maculatus*. *An. maculatus* has the largest number of individuals.

Gia Lai province has collected 12 Anopheles species, of which 2 main vectors of malaria: *An. dirus* and *An. minimus*; and 3 secondary vector species, *An. aconitus, An. jeyporiensis* and *An. maculatus*. In Gia Lai species *An. dirus, An. aconitus,* and *An. maculatus* have the largest number of individuals.

Dak Lak province collected 10 species of Anopheles, including the main vector of malaria *An. dirus*; and 2secondary vector species: *An. aconitus and An. maculatus*.

Table 1: Composition of Anopheles mosquitoes in study sites in Central Region and Central West Highlands

N°	Anopheles mosquitoes	Central Region				Central West Highlands	
		Binh Đinh	Khanh Hoa	Ninh Thuan	Quang Binh	Gia Lai	ĐacLak
1	***An. aconitus*Donitz, 1902***	++++	+	++	+	+++++	+
2	*An. annularis* Van der Wulp, 1884	+	-	+	+	-	-
3	*An. barbirostris* Van der Wulp, 1884	+	+	-	+	+	+
4	*An. crawfordi* Reid, 1953	++	+	-	+	-	-
5	***An. dirus* Peyton et Harrison, 1979****	+	+++++	+++	-	+++++	+
6	*An. jamesi* Theobald, 1901	+	+	+	-	-	-
7	***An. jeyporiensis* James, 1902***	+	-	+	-	++	-
8	*An. kawari* James,1903	-	-	+	-	-	+
9	*An. kochi*Doenitz,1901	+	+	+	+	+	-
10	***An. maculatus* Theobald,1901***	+++++	+++++	+	+++	+++++	+
11	***An. minimus* Theobald,1901****	+	-	+	+	+++	-
12	*An. nivipes* Theobald, 1903	-	-	-	-	+	-
13	*An. philippinenis* Ludlow, 1902	++++	+++	++	+	-	-
14	*An. peditaeniatus* Leicester, 1908	+++	+++++	+	++	+++++	++
15	*An. sinensis* Wiedemann,1882	+++	++++	+	+	++	+
16	*An. splendidus* Koidzumi, 1920	++++	-	++	+	-	+
17	*An. tessellates* Theobald,1901	-	-	-	-	+	+
18	*An. vagus* Doenizt, 1902	+++	+++	+	++	+++	++
	Total	**15**	**11**	**14**	**12**	**12**	**10**

*** Main malaria vector ; * Secondary malaria vector*
+ From 1 to 20 individuals; ++ From 21 to 50 individuals; +++ From 51 to 100 individuals;
++++ From 101 to 200 individuals; +++++ >200 individuals

Distribution of malaria vectors by region

Table 2: Distribution of malaria vectors in Central Region and Central West Highlands

Vector malaria	Central Region						Central West Highlands			
	Quang Binh	Binh Dinh	Khanh Hoa	Ninh Thuan	Number	(%)	Gia Lai	Đac Lak	Number	(%)
An. aconitus	1	131	6	45	**183**	**40.8**	254	12	**266**	**59.2**
An. dirus	0	5	1004	54	**1063**	**52.9**	946	2	**948**	**47.1**
An. jeyporiensis	0	2	0	4	**6**	**18.8**	26	0	**26**	**81.2**
An. maculatus	62	201	295	3	**561**	**51**	522	16	**538**	**49**
An. minimus	7	12	0	2	**21**	**25.3**	62	0	**62**	**74.7**

Table 2 shows that there are 5 malaria vectors in Central Region and Central West Highlands, including the 2 main vectors of malaria*An. dirus, An. minimus* and 3 secondary vectors *An. aconitus, An. jeyporiensis,* and *An. maculatus.* In Central Viet Nam, *An. dirus*(52.9%) is present in similar proportion than in the Central West Highlands (47.1%); *An. minimus* in the Central Region (25.3%)is present in lower proportion than in the Central West Highlands (74.7%); the percentage of secondary vectors*An. aconitus* (40.8%) and *An. jeyporiensis* (18.8%) in the Central Region is lower than in the Central West Highlands,*An. aconitus* (59.2%), *An. jeyporiensis* (81.2%). Lastly, the population of *An. maculatus* in Central Region(51%) is almost equivalent to that of the Central West Highlands (49%).

Distribution of malaria vectors by habitat of the study area

Table 2.1: Distribution of malaria vectors in forest

Vector malaria	At the forest edge						In the forest			
	Quang Binh	Binh Dinh	Khanh Hoa	Ninh Thuan	Number	(%)	Gia Lai	Đac Lak	Number	(%)
An. aconitus	1	131	12	45	**189**	**42.1**	254	6	**260**	**57.9**
An. dirus	0	5	2	54	**61**	**3**	946	1004	**1950**	**97**
An. jeyporiensis	0	2	0	4	**6**	**18.7**	26	0	**26**	**81.3**
An. maculatus	62	201	16	3	**282**	**25.7**	522	295	**817**	**74.3**
An. minimus	7	12	0	2	**21**	**25.3**	62	0	**62**	**74.7**

The ratio of main vectors *An. dirus* and *An. minimus* in forest habitat (*An. dirus*: 97%; *An. minimus*: 74.7%)is higher than in the forest edge habitat (*An. dirus*: 3.03%, *An. minimus*: 25.3%). The rate of secondary vectors, *An. aconitus, An. jeyporiensis,* and*An. Maculatus* in forest habitat is also higher than in the forest edge habitat.

Percentage of malaria infected mosquitoes at study sites

In the malaria vector sites in the Central Region and Central West Highlands, we selected 2 sites todetermine the malaria infection rate of the mosquitoes in order to bring additional elements regardingthe disease transmission in Khanh Hoa and Gia Lai. A total of 2,158specimens belonging to species known to be malaria vectorswere taken in the 2severe malaria endemic places Khanh Hoa and Gia Lai,and have been tested with ELISA to detect mosquito malaria parasites.

Table 3: Number of mosquitoes infected with parasites in Khanh Hoa and Gia Lai

Province	Region	Malaria vector	Number	Malaria parasites (+)	(%)	(+) P.f	(%)	(+) P.v	(%)
Khanh Hoa	Khanh Phu	*An. aconitus*	4	0	0	0	0	0	0
		An. dirus	195	4	2.05	1	**0.51**	3	**1.54**
		An. maculatus	76	0	0	0	0	0	0

	Son Thai	*An. aconitus*	2	0	0	0	0	0	0
		An. dirus	384	4	1.04	0	0	4	**1.04**
		An. maculatus	154	0	0	0	0	0	0
Total			**815**	**8**	**0.98**	**1**	**0.12**	**7**	**0.86**
Gia Lai	Ia Mlah	*An. aconitus*	122	0	0	0	0	0	0
		An. dirus	303	3	0.99	1	**0.33**	2	**0.66**
		An. maculatus	203	0	0	0	0	0	0
	Chư R Căm	*An. aconitus*	132	0	0	0	0	0	0
		An. dirus	249	2	0.8	0	0	2	**0.8**
		An. jeyporiensis	22	0	0	0	0	0	0
		An. maculatus	250	0	0	0	0	0	0
		An. minimus	62	7	11.3	7	**11.3**	0	0
Total			**1.343**	**12**	**0.89**	**8**	**0.6**	**4**	**0.3**

The results of ELISAtesting of 815 mosquitoes in Khanh Hoa district, including 3 species known as major vectors of malaria,are shown in table 3. Total malaria parasite infection rate is 0.98%, of which only the main vector *An. dirus* in both Khanh Phu and Son Thai is infected by*P. falciparum* malaria parasite at 0.51% and *P. vivax* infection from 1.04 to 1.54%. Other vector species have not been found to be infected with the parasite.

The results of ELISA testing of 1,343 mosquitoes in the Gia Laidistrict reveal 5 species asmalaria vectors (Table 3). Mosquitoes of this area are infected by themalaria parasite at a prevalence of 0.89% . In Chu R Cam, *An. minimus* infection with *P. falciparum*is at a very high rate of 11.3%; while*An. dirus*are infected by*P. vivax* at a rate of 0.8%.In Ia Mlah, *An. dirus*are infected by *P. Falciparum* at 0.33% and by *P. vivax* at 0.66%.Other vector species have not been found to be infected with the parasite.

Discussion

Malaria is one of the most deadly and widespread parasitic disease in the world [2]. Many attempts have been made to control, prevent, or even eliminate the disease. One way of fighting it is to study the behavior and species diversity of Anopheles mosquito, the vector of malaria [4]. This field of research is considered important because it could identify the specific species of Anopheles responsible for malaria transmission from human to human or animal to human and vice versa [6]. It could also contribute to malaria prevention by matching the preventive programs to predominant Anopheles species within the area [4].

In Central Viet Nam and Central West Highlands, respectively 16 and 14 species of Anopheles were collected. In both sites, both malaria vectors*An. dirus* and *An. minimus* were found. In the Central Region,the*An. dirus* species is the one present in greaterproportion while in the Central West Highlands *An. dirus* and *An. minimus* speciesare present in equal proportions. In addition, in both study areas, the secondary vectors, *An. aconitus*, *An. jeyporiensis* and *An. maculatus*, were also collected.The distribution of the vectors is also not the same in all regions. Our study thus show that *An. minimus*is present in lesser proportion in the Central Region (25.3%) than in the Central West Highlands (74.7%).

However, there are areas where this species does not appear. According to Chen (2002) and Foley (2008), *An. minimus* is almost absent from northern Chinaand only present in the southern part of the country, including Yunnan, Guangxi, Guangxi, Taiwan and Hai Nam [12-13]. Other studies also suggest that *An. minimus* is not present, or in very little number, in Indonesia and Philippines, while *An. flavirostris* (a member of the *An. minimus* group) is found in these archipelagos [14]. *An. flavirostris*has also been identified as a malaria vector in some West Asian countries such as Nepal or Pakistan [15].

To the west of Viet Nam, *An. minimus* is present in Uttar Pradesh and Andhra Pradesh states (India). *An. minimus* is also defined as the most important malaria vector in the Himalayan foothills [16]. In Myanmar, *An. minimus*is present in almost every territory, and is more likely to transmit malaria than *An. dirus*[17]. In

addition, *An. minimus* is also present in Northeast Asia, like in Japan. Thus, *An. minimus* has a wide distribution in Asia. In Viet Nam, the distribution of *An. minimus* is stretching from the Viet Nam-China border to the South East.

Regarding the main vector *An. dirus*, several studies from the past decade have confirmed that itincludes many subspecies that have been named after the letters of the alphabet(*An. dirus*A, *An. dirus*B, *An. dirus* C, *An. dirus*D) [18]. They are unequally distributed and found in western India, Southeast Asia, Hainan Island, Taiwan. *An. dirus* A is present in central and northeastern Thailand. *An. dirus* D is also found on the Thai-Myanmar border, northwest of Peninsular Malaysia. *An. dirus*C is located in the East of Peninsular Malaysia while*An. dirus*E has been found in India and *An. dirus* F is present only at the Thailand-Malaysia border [18]. According to the distribution map of Valerie Obsome *et al.* (2007), *An. dirus* is present in most of the study sites in Viet Nam[19]. In our study, we showed that the*An. dirus*population was almost equally distributed between Central Region and Central West Highlands since they respectively account for 52.9% and47.1% of the total population counted.

Some Anopheles species are widely distributed, but they are not considered as a main malaria vector in all areas. *An. maculatus* is widely distributed in Southeast Asia but it is only considered as a main malaria vector in Malaysia and some parts of Lao [20].Although habitat changed a lot in recent years, *An. maculatus* is still present in great number. In Viet Nam, *An. maculatus* is a secondary malaria vector but since it is the main malaria vector in some neighboring countries, it is important to include them in prevention measures. In our study, we showed that*An. maculatus*are present in almost equal numbers in Central Region and in Central West Highlands (respectively 51% and 49%).

Morever, there are also secondary vectors such as *An. aconitus* and *An. jeyporiensis*, whose density in each region is different. Secondary vectors*An. aconitus*and *An. jeyporiensis* are present in greater number in the Central Region than in the Central West Highlands (respectively 40.8 and 59.2%, and 18.8 and 81.2%). Morever, *An. Minimus* are more numerous at the survey sites in the forest (74.7%) than at the forest edge (25.3%). In Thailand, *An. minimus* is one of the main vectors of malaria [21]. In Viet Nam, although the environment has changed and chemicals have been used continuously for health and agricultural purposes for a long time on a large scale, *An. minimus* is still present in most of the survey sites in the midland and mountainous areas of the country, especially in the North. Our results are similar to those of Vu Duc Chinh regarding the distribution of malaria vectors for years 2003-2012, in which *An. minimus*were collected in 119 regions and all forests of the country [22].The level of malaria transmission varies from the forest to the forest edge, and to the area away from the forest [23]. InViet Nam, *An. dirus* has been identified as the major vector of transmission of malaria in the mountainous forest areas from North to South. Its distribution is closely related to the distance to the forest: the greater it is, the lesser are the mosquitoes [24].All these results explain why the population living in the forest is more exposed than the population living at the forest edge.

Conclusion

We collected 16 species of Anopheles in Central Viet Namand 14 in Central West Highlands. In both sites, malaria vectorsare *An. dirus* and *An. minimus. An. dirus* species is present in similar proportions in both regions while only one quarter of*An. minimus*was counted in the Central Region and the rest of it inthe Central West Highlands. In addition, the secondary vectors*An. aconitus*, *An. jeyporiensis* and *An. maculatus* were also collected in these areas. The extent of malaria transmission in an area is closely related to the number of vector species present, their density, longevity, and the probability of transmission of the malaria parasite.Further research is thus needed to investigate the Plasmodium infestation rate among these Anopheles species in order to complete the map of species distribution and susceptibility areas in Viet Nam.

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