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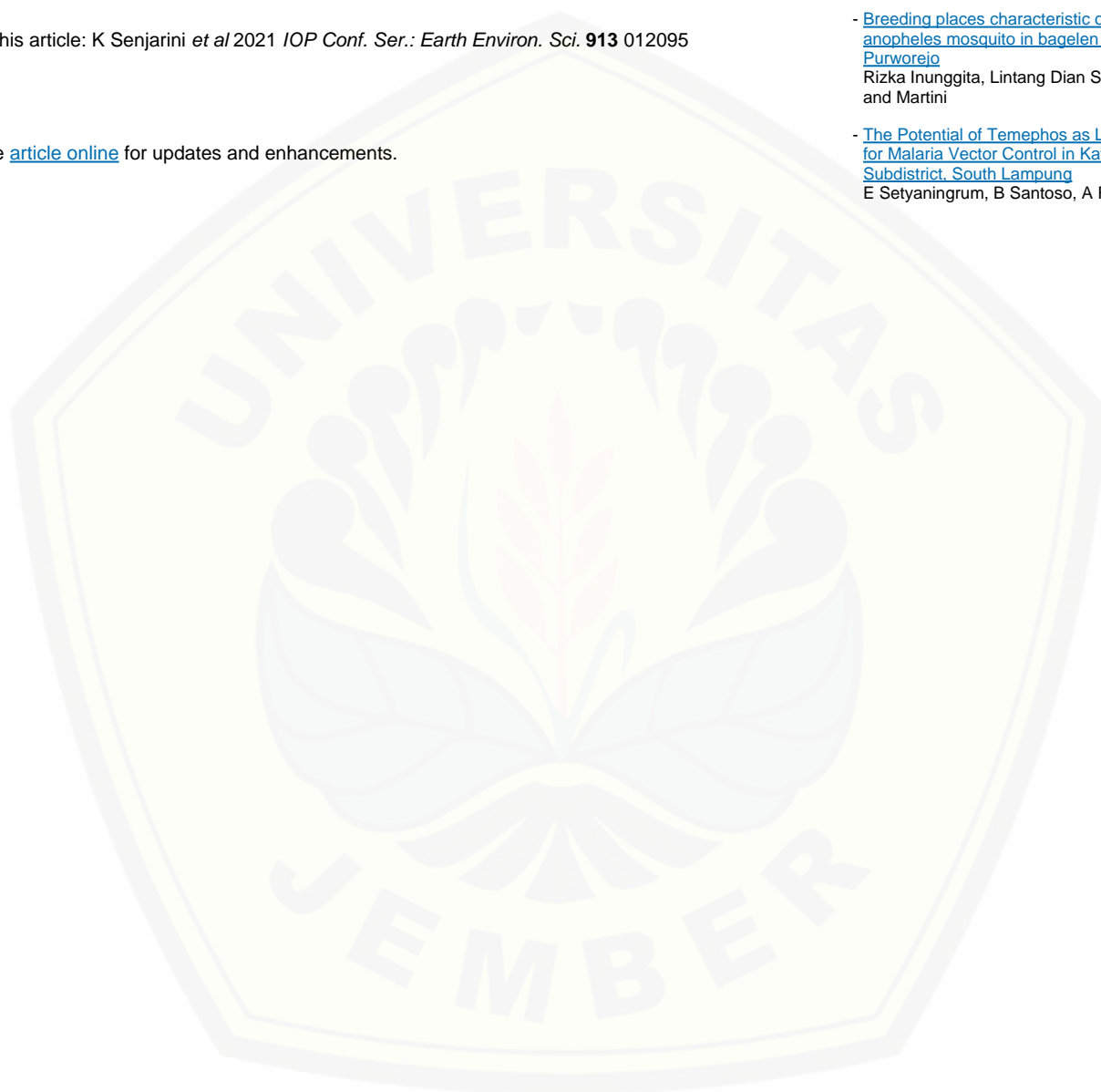
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## Species shifting composition of the *Anopheles* vector in Wongsorejo district - Banyuwangi, Indonesia

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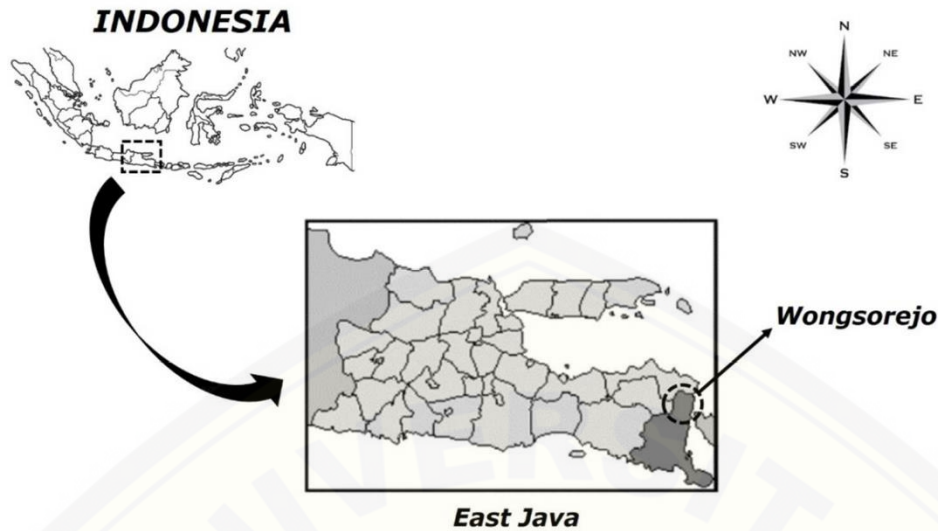
**Abstract.** Malaria's cases have been reported to occur annually in Wongsorejo district of Banyuwangi, East Java since 2002. However, there is a significant decrease of malaria cases during these last years, which might be related to the malaria vector species shifting composition. The objective of this research was to observe some important bionomic characteristics of malaria vector *Anopheles* in this area from 2015 until 2020 which include species identification, blood feeding behaviour and biting preference of vector. The data collection i.e. determination of *Anopheles* diversity and behaviour was conducted monthly for 3-6 months annually from 2015 – 2018. In 2019-2020 we sampled irregularly to see the trend. The results showed that there were species shifting of *Anopheles* vectors in this area. The proportion of *Anopheles (An.) sundaicus* and *An. subpictus*, which were previously reported until 2015 as the main *Anopheles* species in this area, significantly decreased in 2016 - 2018. *An. indefinitus* & *An. vagus* was becoming the majority of *Anopheles* species. During 2019-2020, *An. vagus* became the main species identified in the sampling area. The predominant species of *An. vagus* and *An. indefinitus* has exophagic and zoophilic preference behaviour. Furthermore, *An. indefinitus* has not previously been identified as a vector for malaria, compared to *An. sundaius*, and *An. subpictus* which are well known as an important primary malaria vector on Java Island, Indonesia.

### 1. Introduction

Malaria remains an important health threat in rural areas of Southeast Asia including Indonesia. Many attempts had been made to control, prevent, and even eliminate the disease. Studying diversity and behaviour of *Anopheles (An.)* mosquito, which is the vector of malaria, plays a crucial role in developing strategy for an effective and successful vector control program to combat malaria transmission [1]. The exploration and mapping of *Anopheles* species and its distribution are also important as Indonesia is an archipelago country with each of its islands or regions having very distinct ecological characteristics from one another.

Wongsorejo district, especially the Bangsring area is a coastal region in Banyuwangi, East Java Indonesia, which has been identified as a malaria-endemic area since 2002 [2]. The highest incidence of malaria was reported in 2011 with 127 cases as recorded at Wongsorejo Centre for Public Health (2013). Previous studies on bionomic of vectors and its diversity in Wongsorejo Banyuwangi showed that *An. sundaicus* was the main dominating species, however many other species vectors were also identified [2]. The objective of this research was to observe some important bionomic characteristics of malaria vector *Anopheles* in this area (Figure 1) from 2015 until 2020, which include species identification and its structural composition, blood feeding behaviour and biting preference.





**Figure 1.** Sampling site location for *Anopheles* collection.

## 2. Methods

Female adult mosquitoes were collected monthly using hand-held aspirators for six to seven months annually from 2015-2016 (November-April), 2016-2017 (November-April) and 2017-2018 (November-January). To observe further trends of species composition, additional irregular samplings (three times a year) were also conducted between in 2019 and 2020. Sampling was conducted every hour for 12 hours between 06.00 p.m. – 06.00 a.m. Human landing and light-trap collections were made indoors and outdoors as well as around cattle shelters. The mosquitoes were then placed inside a labelled paper cup, identified and counted to species level by morphological characters using taxonomical book [3] and was also compared with the book of *Anopheline Mosquitoes of Malaya & Borneo* [4]. All data were descriptively analysed to see the diversity of the species as well as its biting behaviour.

## 3. Results & Discussion

Total mosquitoes collected throughout the sampling period were 3723 which consisted of five species of *Anopheles* i.e: *An. vagus*, *An. barbirostris*, *An. subpictus*, *An. sundaicus* and *An. indefinitus*. Compared to the previously published paper from our research group [5], there was a decreasing tendency of the total number of mosquitoes from year to year (Table 1.) Previous results showed that *An. sundaicus* and *An. subpictus* were the main species in 2014 [5], however their numbers significantly decreased in the 2015-2020. *An. vagus* and *An. indefinitus* were thereafter becoming the majority of *Anopheles* species in this area.

In this study, we also elaborate the biting behaviour of collected *Anopheles* mosquitoes based on the number of mosquitoes which were resting on human and/or cattle/cattle shelter during sampling. All collected mosquitoes are predominately zoophilic, exophagic and exophilic species. Only one mosquito was found indoor at the position of human resting in 2014 [5], the remaining samples were outdoor and predominantly zoophilic. In 2016-2020, there were even no mosquitoes resting on human structure (Table 2). From all of the observation period, the highest peak of mosquito's activities was from 18.00-19.00. It remained consistent until 23.00 but decreased starting from 24.00. The last *Anopheles* mosquito's activity was detected between 05.00 - 6.00 a.m.



**Table 1.** Species mosquito's composition during observation period of 2015 – 2020 in Wongsorejo district, Banyuwangi.

Name of Species	Sampling Period				
	2014 <sup>[5]</sup>	2015-2016	2016-2017	2017-2018	2019-2020
<i>An. vagus</i>	8%	15%	50%	64%	72%
<i>An. barbirostris</i>	1%	1%	0%	0%	1%
<i>An. subpictus</i>	6%	1%	3%	5%	8%
<i>An. sundaicus</i>	83%	23%	8%	2%	17%
<i>An. indefinitus</i>	1%	61%	39%	29%	2%
Total number of collected mosquitoes	2.961	1.919	933	543	328

Among 5 *Anopheles* species collected from this study, only *An. indefinitus* that has not been confirmed as malaria vector in Indonesia. *An. vagus*, *An. barbirostris*, *An. subpictus*, and *An. sundaicus* from Indonesia have been reported to be positive infectious considering the presence of salivary gland sporozoites [6]. From the species composition, this study showed also that there was a decreasing number of *An. sundaicus* and *An. subpictus* which was accompanied by an increasing population of *An. indefinitus* and *An. vagus*. This change of species domination indicated a species shifting composition of *Anopheles* in this area. The species shifting composition in latitude or elevation could be in response to changing climate with a wide diversity of responses by individual species [7]. However, in addition to the abiotic factors, such as temperature and humidity, there was no significant change in those environmental conditions during the observation period. The lowest temperature and humidity recorded by our thermohygrometer were 23.6°C and 59.3(%RH), whereas the highest were 30.8°C and 91.2(%RH), respectively. Therefore, the changes in the species composition of vectors is very likely in response to human vector-control interventions around observation areas [8]. This commonly occurs when the vector species that are more sensitive to a specific vector-control measure become less common, leaving vector species that are less sensitive.

There are already many studies about *An. sundaicus* and *An. subpictus* being the main *Anopheles* species in coastal areas in Indonesia [9]. Among those 4 potential vectors of malaria in this area, *An. sundaicus* is mainly responsible for malaria transmission in the coastal area of Indonesia. This has been related with its slightly anthropophilic tendency while *An. subpictus*, *An. barbirostris* and *An. vagus* are predominately zoophilic, exophagic and exophilic species [6] which were relevant with this study. *An. sundaicus* has been also proven to be more often infected than any other species tested over a 30-year period across Sumatra (Nias, Sihepeng, Riau/Bintan Island, Lampung), Java (Pari Island, near Jakarta) and the Lesser Sunda Islands (Sumbawa, Flores and Adonara Islands) by CSP-ELISA analysis [10]. *An. indefinitus* was previously reported caught only in hilly-rice field areas in Java, associated with grassy pools, ponds and ditches [11]. We found only one report in Sikka, Flores mentioning about the presence of *An. indefinitus* in rice fields and lagoons but with limited numbers and low population density [12].

The high numbers and domination of *An. indefinitus* during three years of our study was unexpected and mainly observed in sampling areas around lagoons. It seems that the decrease of malaria cases is very likely due the change in vector species domination into *An. indefinitus* and *An. vagus*. Although only *An. indefinitus* is identified as a non-malaria vector in Indonesia, however the preference biting behaviour of *An. vagus* in this study were more into zoophilic and exophagic which are also relevant with the previous studies [13, 14]. This was also supported by the fact that there are always domestic animals around the sampling site. Thus, it can be concluded that having a domestic animal around the settlement could attract this species, and hence, decrease the risk of malaria infection (cattle barrier) [15]. This study was also conducted on the peripheral area close to shrub and lagoon which are suitable breeding grounds for salt tolerant Anopheline mosquitoes. This will also provide a natural barrier of malaria transmission in coastal areas such as Wongsorejo.

**Table 2.** Biting behaviour of *Anopheles* mosquitoes throughout the sampling period.

Mosquitoes Activities	Sampling Period				
	2014 <sup>[5]</sup>	2015-2016	2016-2017	2017-2018	2019-2020
<b>Outdoor</b>					
Human Resting/Bates	459	7	0	0	0
Resting in cattle/Animal Bates	2501	1912	933	543	328
<b>Indoor</b>					
Human Resting/Bates	1	0	0	0	0
Total number of collected mosquitoes	2.961	1.919	933	543	328

Furthermore, as mentioned above, the shifting of species composition may be due to vector-control intervention, thus it could also influence the reduction number of endophilic biting vectors (indoors) but increased the proportion of exophilic biting vectors (outdoor) (Table 2). Personal communication with people living around the observation area showed that use of insecticides are commonly applied indoors through spraying, burning or using electric mosquito repellent to avoid mosquito bites. Long-lasting insecticidal nets and indoor residual spraying programs have been also reported previously to be attributed to the change in vector's composition and behaviour [8].

To our knowledge, this is the latest detailed observation on bionomic of *Anopheles* vectors in this area. Although malaria outbreak was reported in Wongsorejo Banyuwangi with 127 cases in 2013 (Report of Dinas Kesehatan Banyuwangi 2010-2013), however since 2015, this city is declared as malaria free area (Official website of Banyuwangi city). Considering the geographical position of Banyuwangi as a junction border between Java and East Timur Indonesia (Bali and Nusa Tenggara), therefore migration of people between those areas also plays an important factor in the emergence of imported malaria [16]. However, apart from this fact, the species shifting composition of malaria vectors in this study should be also considered to give significant impact on malaria transmission.

#### 4. Conclusion

There was a species shifting composition of *Anopheles* in the sampling site from 2015-2020. The previously reported main malaria vectors have been significantly decreased during the observation period. The predominant species of *An. indefinitus* and *An. vagus* has exophagic and zoophilic preference behaviour. This could be an important factor in the significant decrease of malaria cases in this area during these last years.

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