Determination of mosquito diversity using 3D digital microscope in Cibinong district, Bogor Regency

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Abstract. Bogor Regency is a suburban area with hilly topography and very wet tropical climate. In addition, there are still lot of bushes and the location is crossed by river basin. These conditions create an ideal habitat for mosquitoes, which are the deadlest animal in the world. Despite the high incidence of vector-borne diseases caused by mosquitoes in this area, the information regarding mosquito diversity in Cibinong District, Bogor Regency remains limited. The aim of this study is to determine mosquito diversity in Cibinong District, Bogor Regency. In this study, mosquitoes were collected using human landing catch and sweep nets. Then identification was performed using 3D digital microscope. The study’s findings revealed the presence of eight mosquito species from two genera: Aedes and Culex. In conclusion, this study provides valuable insights into the ecology and diversity of each collected mosquitoes.

1 Introduction

Cibinong is a suburban area and capital of Bogor Regency, West Java, Indonesia, located immediately south of Depok city and becoming satellite town of Jakarta metropolitan area [1]. This area has a tropical rainforest climate with heavy to very heavy rainfall year-round. Furthermore, it has high humidity due to the tropical climate make it an ideal breeding ground for mosquitoes [2].

Mosquitoes are insects that can transmit various diseases to humans and animals through their bites [3]. Some of the most common mosquito-borne diseases include malaria, dengue, chikungunya, Zika, and West Nile virus [3]. These diseases can cause significant morbidity and mortality, especially in tropical and subtropical regions [4]. Therefore, it is important to identify the different types of mosquitoes that can carry and spread these pathogens, and to implement effective strategies to control their populations and prevent their bites [5].

Mosquito identification involves using morphological, molecular, or ecological methods to distinguish between different species or populations of mosquitoes [6]. It can help determine the distribution, abundance, and diversity of mosquitoes in a given area, as well as their potential role in disease transmission [7]. Also, it helps to monitor the emergence and

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spread of insecticide resistance among mosquitoes, which can affect the efficacy of mosquito control interventions [5].

In addition, mosquito identification and control also can have significant impacts on reducing disease transmission and improving human health. For example, a study in India showed that a large-scale mosquito control program that combined source reduction, larval control, and adult control reduced the incidence of malaria by 88% in five years [8]. Another study in Brazil showed that a community-based mosquito control program that involved removing potential breeding sites and applying larval control reduced the incidence of dengue by 74% in one year [9].

Precise identification of mosquitoes can be utilized to ascertain their conduct, environmental impact, significance in spreading diseases, and tactics for controlling them. Ecological behaviour encompasses feeding site, biting habits, geographic range, and ability to act as a vector [10]. The aim of this study is to identify mosquito diversity in Cibinong district, Bogor Regency.

2 Materials and method

2.1 Mosquito collection

Mosquito collection was carried out in Cibinong District, Bogor Regency, West Java, Indonesia. The mosquito collection sites were near residential and office areas, using the human landing catch method and sweep net. Mosquitoes were collected during their peak biting activity, which occurred between 6 PM and 6 AM on September 2023. Subsequently, the mosquitoes were exterminated by putting them in the refrigerator and freezing for 30 minutes [11].

2.2 Mosquito identification

The mosquitoes that had been collected were then observed using 3D digital microscope Keyence VHX-6000® with 100-250x magnification in iLab building, National Research and Innovation Agency (BRIN), Bogor Regency, West Java, Indonesia. Mosquitoes with missing sections of their bodies that could not be recognized were eliminated. Samples identification was according to established identification keys [12,13].

3 Results and discussion

In this study, mosquitoes were collected from two sites with different elevation in Cibinong district, Bogor Regency, West Java, Indonesia (Table 1.). The first site was at the residential area, which has empty land behind it and near to small drain. While the second site was at the office area, which still has a lot of bushes and a small river near it.

<table>
<thead>
<tr>
<th>Collection site</th>
<th>Name of sub-district in Cibinong district, Bogor Regency</th>
<th>GPS coordinates</th>
<th>Elevation (m)</th>
<th>Collection date</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pakansari, Cibinong</td>
<td>N 01°02’20.43” E 124°28’26.45”</td>
<td>115</td>
<td>September 17-19&lt;sup&gt;th&lt;/sup&gt;, 2023</td>
</tr>
<tr>
<td>2</td>
<td>Cibinong, Cibinong</td>
<td>N 01°02’20.43” E 124°28’26.45”</td>
<td>128</td>
<td>September 24-26&lt;sup&gt;th&lt;/sup&gt;, 2023</td>
</tr>
</tbody>
</table>
Mosquitoes are top killer animal in the world and about one million people die each year due to the diseases they carry [9]. They can transmit disease such as Zika virus, Malaria, Dengue, West Nile virus, and Japanese Encephalitis through bites [9]. In this study, mosquitoes in Cibinong district, Bogor Regency were collected and identified to see their diversity. The presence and number of mosquitoes at the sampling sites area may provide a danger for disease transmission to humans.

A total of 31 mosquitoes were collected during the designated time period. Subsequent analysis and identification revealed that among these specimens, 30 mosquitoes were classified as Culex mosquitoes, while one mosquito belonged to the Aedes genus (Table 2.). Table 2 shows that mosquitoes collected consisted of eight species among others: *Ae. albopictus*, *Cx. quinquefasciatus*, *Cx. tritaeniorhynchus*, *Cx. bitaeniorhynchus*, *Cx. fuscocephalus*, *Cx. pseudosinensis*, *Cx. diegensis*, and *Cx. sinensis*.

**Table 2.** The list of mosquitoes identified in this study.

<table>
<thead>
<tr>
<th>Site</th>
<th>Genus</th>
<th>Species</th>
<th>Total</th>
<th>Sex</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Male</td>
</tr>
<tr>
<td>1</td>
<td><em>Aedes</em></td>
<td><em>albopictus</em></td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>quinquefasciatus</em></td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>tritaeniorhynchus</em></td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>bitaeniorhynchus</em></td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td><em>Culex</em></td>
<td><em>fuscocephalus</em></td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>pseudosinensis</em></td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>diegensis</em></td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>sinensis</em></td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td><em>Culex</em></td>
<td><em>pseudosinensis</em></td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>sinensis</em></td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>fuscocephalus</em></td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>TOTAL</strong></td>
<td><strong>31</strong></td>
<td></td>
</tr>
</tbody>
</table>

Observations using a 3D digital microscope Keyence VHX-6000® with 100-250x magnification makes it easier and gives more detail to identify morphological features of mosquito species (Fig 1.). This is different from the research conducted by Supriyono et al. and Chaturanga et al. [7,11]. An advanced light microscope Leica M205Câ were used for morphological identification by Supriyono et al., while Chaturanga et al. use a dissecting microscope. In a wide range of biological studies, it is highly desirable to visualize and analyse three-dimensional (3D) microscopic images. Visualization and analysis methods are critical for understanding and using 3D microscopic images for various cell biology, structural biology, neurosciences, and systems biology applications [14]. A 3D microscope, also known as a stereomicroscope or a dissecting microscope, is an optical microscope designed for three-dimensional observation of objects. Unlike compound microscopes, which are primarily used for viewing thin, transparent specimens in two dimensions, 3D microscopes provide a stereo or three-dimensional view of thicker, opaque objects, allowing the viewer to see the specimen with depth and perspective [15]. 3D imaging has provided new biological insight, as well as pointing out the advantages and limitations of each technique.

This study showed that Culex mosquito had the most diverse species in two sub-district of Cibinong district, Bogor Regency, West Java. *Cx. fuscocephalus* is the most abundant species collected in this study (35.48%) compared to others. This mosquito species was caught mostly near residential areas. These findings are similar to those reported by Chaturanga et al. and Garjito et al. [7,16]. Male *Cx. fuscocephalus* were also collected at the second site (Fig 2.). *Cx. fuscocephalus*, *Cx. tritaeniorhynchus*, *Cx. quinquefasciatus*, and *Cx. vishnui* have all been identified as vectors of Japanese encephalitis (JE) [17]. Japanese
encephalitis has been identified as the leading cause of viral encephalitis in numerous Asian nations [18]. In Bali, the annual occurrence rate of JE is 7.1 per 100,000 children under the age of ten [19]. This mosquito vectors thrive in locations that are surrounded by rice fields, ponds, and pits [20].

Fig. 1. Morphological identification of adult female Aedes albopictus using 3D digital microscope with 200x magnification.

Then, about five Cx. quinquefasciatus (16.12%) were collected in this study. This mosquito species was also reported high abundant in Dramaga district, Bogor Regency, Indonesia, near Macaca fascicularis breeding facilities [21]. Cx. quinquefasciatus has been identified as a primary vector of West Nile virus (WNV) [22]. This virus can cause West Nile fever (WNF) in humans with symptoms include a mild, self-limiting flu-like sickness, in extreme cases (rare) can proceed to encephalitis which can be fatal [23].

Furthermore, two Ae. albopictus (3.22%) were collected near residential area. It is similar to the results reported by Ikhsan et al. that Ae. albopictus larvae are the most abundant in

Fig. 2. Adult male Cx. fuscocephalus were collected and observed under 3D digital microscope with 100x magnification.
residential areas in Bogor City, West Java. [24]. This showed that the main habitat of *Ae. albopictus* is around a residential area. This mosquito species are the secondary vectors of dengue virus that most common in urban area and rural areas [25], they are also found in a variety of habitats across the tropics and subtropics [21]. Their presence with *Ae. aegypti* has negative impacts on public health every year because they can cause dengue fever. According to the Lu *et al.*, dengue cause around 3 million cases across the America, the highest number ever recorded [26].

Successively, one of each *Cx. tritaeniorhynchus*, *Cx. bitaeniorhynchus*, and *Cx. sinensis* were collected with a percentage of 3.22%. *Cx. tritaeniorhynchus* usually bites in outdoor environments, where its main targets are animals [27]. However, if these animals are not present, humans will be the target of their bites [27]. This is also in line with Novianto *et al.* that *Cx. tritaeniorhynchus* was found around long-tailed macaque (*Macaca fascicularis*) breeding facilities [21]. In this study, *Cx. sinensis* was also successfully collected around residential area. This is also similar to study reported by Supriyono *et al.* and Shalihah *et al.* [28] [27]. And finally, a female *Cx. bitaeniorhynchus* was successfully collected. This species is rarely found or recorded in publications. However, this kind of species is known as a vector for Sindbis fever [29].

All findings above may be affected by several factors, among others: high rainfall, geographical characteristics, deforestation, and ecological structures. Bogor area is called a rainy city because it has relatively greater rainfall compared to other areas in West Java. According to Khoirunnisa *et al.* [30], the average rainfall in Bogor area is around 3,000 mm/year. Based on Yulianti *et al.*, high rainfall can affect mosquito diversity in an area because it can increase the number of habitats and water sources for mosquitoes [31]. Then, geographical characteristics also have a large contribution to mosquito diversity. Cibinong district has many small lakes and streams around it that are possible breeding grounds for mosquitoes, including *Situ Cikaret, Situ Cibuntu, Dora Ecopark Lake, Situ Gedong, Situ Citatah, Situ Kibing, Situ Kebantenan, Situ Pemda, and Cibinong Situ Plaza*. Furthermore, deforestation can alter the availability and quality of larval habitats for different mosquito species, as well as the distribution and abundance of their hosts and predators. A study in Brazil found that deforestation increased the abundance of *Anopheles darlingi*, a major malaria vector, by creating more suitable breeding sites [32]. Lastly, spatial and temporal patterns of mosquito population dynamics, which are influenced by various environmental and biological factors. A study in India found that mosquito diversity and abundance varied across seasons and regions, depending on the occurrence of different species complexes and their interactions with climatic variables [33].

### 4 Conclusion

There were eight species of mosquitoes from Culex and Aedes group identified from the sampling site. This research indicates that Cibinong District, Bogor Regency, has quite a diversity of mosquito species. This may be caused by several factors, including high rainfall, geographical characteristics, deforestation, and ecological structures. Since this study was the first to examine mosquito diversity in Cibinong district, Bogor Regency, West Java, Indonesia, more species may be found in the future.

The authors gratefully acknowledge DIPA 2023 from Research Organization for Health, National Research and Innovation Agency (BRIN), Indonesia through *Rumah Program Hasil Pengembangan Alat Dan Deteksi Kesehatan* (No. 16/III.9/HK/2023) for the research financial support. And used the facilities in the Laboratory of Microscopy, iLab – BRIN at Science and Techno Park Soekarno in Cibinong district, Bogor Regency, Indonesia.

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