Spatial distribution pattern of ornithophilic mosquitoes along the vertical axis in forest habitats

W.G.D. Chathuranga, T.C. Weeraratne, S.H.P.P. Karunaratne and W.A.P.P. De Silva*

Highlights

• The abundance of mosquitoes varied along the vertical axis of forest strata.
• Canopy level is preferred by diverse mosquito species and *Culex pseudovishnui* was common at all height levels.
• Generalized feeders are frequent near human settlements.
• Specialized feeders are abundant close to natural forest patches.
Spatial distribution pattern of ornithophilic mosquitoes along the vertical axis in forest habitats

W.G.D. Chathuranga\textsuperscript{1,2}, T.C. Weeraratne\textsuperscript{3}, S.H.P.P. Karunaratne\textsuperscript{1} and W.A.P.P. De Silva\textsuperscript{2}

\textsuperscript{1}Department of Zoology, Faculty of Science, University of Ruhuna, Matara, Sri Lanka.
\textsuperscript{2}Department of Zoology, Faculty of Science, University of Peradeniya, Peradeniya, Sri Lanka.
\textsuperscript{3}Postgraduate Institute of Science, University of Peradeniya, Peradeniya, Sri Lanka.

Received: 13/12/2021; Accepted: 28/07/2022

Abstract: The spatial and temporal distribution of hosts is crucial in determining the feeding behaviour of hematophagous insects. We explored the spatial distribution of ornithophilic mosquitoes along the vertical axis in four locations covered with forests in Sri Lanka, i.e. Hanthana forest reserve (HAN), Halgolla forest reserve (HAL), Peradeniya university park (UOP), and Gampola (GAM). Mosquitoes were collected using bird-baited traps set at different height levels: 1 m (forest floor), 3 m (understory vegetation), and 6 m (canopy level), from 6.00-10.00 p.m. in 20 sampling sessions. Mosquitoes were identified using standard taxonomic keys and DNA barcodes of the cytochrome c oxidase subunit I (COI) gene. A total of 1212 mosquitoes belonging to 15 species and seven genera were collected and the highest numbers were reported from canopy level [HAN (219; 53.3%), HAL (93; 49.5%), GAM (136; 45.5%), and UOP (163; 51.7%)]. Culex pseudovishnui was the only species distributed evenly along the vertical axis. Although the mosquito abundance was significantly different along with three height levels, the diversity was not significantly different. Generalized feeders were common in patchy forests located close to human settlements and more specialized feeders were found close to natural forests. The findings of the study are important in understanding the interaction between ornithophilic mosquitoes with residing avifauna in each forest strata.

Keywords: bird-baited traps; blood-feeding pattern; bird roosting; Culex mosquitoes; forest stratification.

INTRODUCTION

Different vegetative strata in forest ecosystems provide unique ecological niches for diverse faunal groups. Consequently, wild organisms tend to occupy different forest strata with specific morphological, physiological, and behavioral adaptations (Jayson and Mathew, 2003; Tantely et al., 2019). Spatial and temporal distribution along the vertical axis of forest habitats have been reported for mammals (Rogozi et al., 2013), birds (Jayson and Mathew, 2003, Subasinghe et al., 2014), and some arthropods (Ulyshen, 2011). Previous studies have reported the vertical distribution of some arthropod species such as leafhoppers (Meneses et al., 2016), ants (Glaser, 2006), spiders (Pekar, 2005), as well as a few species of mosquitoes (Derraik et al., 2005; Jansen et al., 2009; Diallo et al., 2010). These studies have shown that the vertical distribution of fauna is mainly determined by specific ecological interactions (Hayes and Gruver, 2000; Rohner et al., 2000).

The research works carried out on mosquitoes have shown that some species are evenly distributed from ground level to canopy levels of the forest habitats (Braack et al., 1994), while some are restricted to ground level (Flemings, 1959; Derraik et al., 2005) while some species are to the canopy level (Anderson et al., 2004; Jansen et al., 2009; Diallo et al., 2010; Swanson and Adler, 2010; Tantely et al., 2019). For instance, Aedes punctotboracis, Anopheles melas, An. squamosus, Culex annulirostris, Cx decens, Cx tritaeniorhynchus, Ochlerotatus antiopes, Mansonia africana, and Ma. uniformis are common examples of mosquito species that are frequently reported at the ground level whereas Ae. kochi, Cx asteliae, Cx pervigilans, Cx neavei, Cx weschei are mostly identified at the canopy level. Mosquito species such as Coquillettidia iracunda, Cx pipiens, Cx poicilipes, Cx restuans have shown a broader range of distribution from the ground level to the canopy level (Derraik et al., 2005; Drummond et al., 2006; Jansen et al., 2009; Diallo et al., 2010).

Although the diversity and distribution of Sri Lankan mosquitoes have been widely studied considering their impact on human health (Amerasinghe and Alagoda, 1984; Jayathilake et al., 2015; Chathuranga et al., 2018; Weeraratne et al., 2018), habitat preferences and niche occupancy of Sri Lankan forest-dwelling mosquitoes have been poorly evaluated. Therefore, a proper understanding of niche occupancy and the spatial and temporal distribution of forest-dwelling mosquitoes are timely and important, especially with the increased anthropogenic influences on forest ecosystems. As a result, the invasion of forest-dwelling mosquitoes to human settlements can also be expected giving a significant impact on the transmission of mosquito-borne diseases among human populations and livestock. Ornithophilic mosquitoes represent a group of important mosquitoes that act as vectors of many pathogens including viruses (West Nile Virus, Japanese encephalitis), bacteria (Sphingomonas spp., Rahnella spp.), protozoans (Haemoproteus spp., Plasmodium spp., Leucocytozoon...
spp.), and helminths (Filarial worms) (Valkiūnas, 2005; Ishtiaq et al., 2008; Lequime and Lambrechts, 2014; Zotzmann et al., 2017). The spatial distribution of these mosquitoes may also have influenced the distribution of these mosquitoes. According to our understanding, only a few studies have been carried out to understand the spatial distribution of ornithophilic mosquitoes in different forest strata (Anderson et al., 2004; Derraik et al., 2005; Swanson and Adler, 2010), and no such studies have ever been taken place in Sri Lanka. Thus, in this study, we investigated the spatial distribution of forest-dwelling ornithophilic mosquitoes along vertical strata of four different forest habitats in central Sri Lanka. The findings of the study are crucial in understanding the specific interactions between mosquitoes and avifauna in tropical forest strata.

MATERIALS AND METHODS

Study sites

The vertical distribution of ornithophilic mosquitoes was studied in four areas covered with forests in the Kandy district of central Sri Lanka (Figure 1); i.e. Gampola (GAM) (7.165281N, 80.573407E), Peradeniya University Park (UOP) (7.259740N, 80.597462E), Halgolla Forest Reserve (HAL) (7.306687N, 80.522264E), and Hanthana Forest Reserve (HAN) (7.249775N, 80.613157E). HAN and HAL are secondary natural forests located around 1 km away from human habitation while UOP and GAM are patchy forests located close to human settlements.

Mosquito collection

Bird-baited traps were used to capture ornithophilic mosquitoes at three different height levels, i.e. 1 m, 3 m, and 6 m above the ground level, in the study sites. These height levels represent the forest floor (1 m), understory vegetation (3 m), and canopy level (6 m), which are common nesting and roosting sites of avifauna. A total of 20 sampling sessions were conducted between January to May 2019 covering five sampling sessions per site. Traps were placed from 18:00 h to 22:00 h, the period which was found to be the most active time for ornithophilic mosquitoes (Chathuranga et al., 2018). In one sampling session, three bird-baited traps and a control trap were placed at each height level maintaining at least a 50 m horizontal distance between two traps. These traps were a modified version of the CDC miniature light trap with a cubic animal compartment to place the bird (20×20×20 cm) and one to three-month-old domesticated female Japanese quails (Coturnix japonica) was used as the bait. Animal ethics approval was obtained from the ethics committee of the Postgraduate Institute of Science (approval number: ECC_PGIS_2017_05), University of Peradeniya, Sri Lanka. Control traps were placed without a bird. Collected mosquitoes were brought to the Vector Biology Research Laboratory (VBRL) of the Department of Zoology, University of Peradeniya, Sri Lanka, and preserved at −4 °C until use.

Identification of mosquitoes

Adult mosquitoes were identified morphologically using standard taxonomic keys (Barraud, 1934; Amerasinghe, 1995). DNA barcoding was performed to confirm the identity of mosquito species that had uncertainties in morphological identifications. DNA was extracted from individual mosquitoes following the protocol described by Livak (1984). The mitochondrial cytochrome c oxidase I (COI) gene of the mosquitoes was amplified using the primers, C1-J-1718F (5'-GGA GGA TTT GGA AAT TGA TTA GTT CC-3') and C1-N-2191R (5'-CCC GGT AAA ATT AAA ATA TAA ACT TC-3') (Simon et al., 1994). PCR amplifications were done in a thermal cycler (Techne-Flexigene, England) in 25 μl reaction volumes. Each 25 μl PCR reaction contained 7.5 μl GoTaq® Green Master Mix (Promega, USA), 1.0 μl each from forward and reverse primers (Macrogen, Korea), 5 μl DNA templates, and 10.5 μl nuclease-free water (Promega, USA). The PCR cycling conditions were: initial denaturing at 95 °C for 3 min, followed by 30 cycles of 94 °C for 1 min, 55 °C for 1 min, and 72 °C for 1 min. The final elongation was done at 72 °C for 10 min, while the final holding was done at 4 °C (Nolan et al., 2007). Five microliters of the PCR products were run on 1.5% agarose gel with a 100 bp standard DNA size marker (Invitrogen, California, USA) to verify the PCR amplification. Samples with a DNA band of around 480 bp size were scored as positive. The PCR products that gave positive results were sequenced using an automatic DNA sequencer (Applied Biosystems series 3500, U.S.A.) at the Department of Molecular Biology and Biotechnology, University of Peradeniya, Sri Lanka. The DNA sequences were annotated using the GenBank (https://www.ncbi.nlm.nih.gov/) database and blastn tool.
Statistical analyses

The Margalef’s Index (d) and Pielou’s Evenness (J’) were used to characterize species diversity in the three different height levels of selected habitat types. The similarity of height levels in terms of abundance and distribution of ornithophilic mosquitoes were compared by making a Bray-Curtis similarity matrix using Primer-E 7 software (Clarke and Gorley, 2015). Since the data were normally distributed (Normality Test-Anderson-Darling, \( p < 0.05 \)), a one-way ANOVA test was performed to determine the variations in the vertical distribution of ornithophilic mosquitoes. All statistical analyses were carried out using MINITAB 14 statistical software and at a significance level at \( p < 0.05 \).

RESULTS AND DISCUSSIONS

A total of 1,212 mosquitoes belonging to seven genera (Aedes, Armegeres, Coquilletidia, Culex, Mansonia, Mimomyia, Orthopodomyia), and 15 species were collected (Table 1). Of them, the identification of two individuals of Mimomyia chamberlaini and one individual of Cx fuscanus were confirmed using the DNA barcoding results. No mosquitoes were found in the control traps set at all three height levels in four different study sites, confirming the zoophilic nature of these mosquitoes and the preference for bird blood.

Diversity of ornithophilic mosquitoes

The majority (eight species) of the collected mosquito species belonged to the genus Culex and they dominated all three height levels (Figure 2).

Genus Culex has been recognized as the most abundant ornithophilic mosquito species (Meister et al., 2008; Jansen et al., 2009; Molaei et al., 2010). According to Chathuranga et al. (2018), 13 Culex mosquito species in Sri Lanka preferentially feed on bird blood. However, the present study added two more Culex species, i.e. Cx vishnui and Cx (Culicomyia) bailyi to the previous list. The highest number of species were reported at the canopy level (6 m) from all four study sites [HAN (n = 10), HAL (n = 6), UOP (n = 8), GAM (n = 5)] (Figure 2). Out of the total collection of 15 species, Mansonia annulifera was restricted mainly to the canopy level (6 m) of HAN while Cx sitiens, Cx fuscanus and Mimomyia chamberlaini were limited only to the canopy level of UOP (Table 1).

Culex pseudovishnui was dominated in the HAN (37.5%) and HAL (42.0%) forests where they were distributed evenly along the vertical axis of the vegetation. However, Cx nigropunctatus was the most common mosquito species at UOP (33.0%) and GAM (57.2%) which showed a nearly equal vertical distribution along the forest strata. Figure 3 shows the distribution of ornithophilic mosquitoes at three height levels in four selected habitats.

Figure 2: Number of ornithophilic mosquito species reported at three height levels of selected forest habitats.
Table 1: Abundance and diversity of Ornithophilic mosquitoes collected from four different study sites at three height levels using bird-baited traps.

| Species                   | HAN 1 m | HAN 3 m | HAN 6 m | HAL 1 m | HAL 3 m | HAL 6 m | UOP 1 m | UOP 3 m | UOP 6 m | GAM 1 m | GAM 3 m | GAM 6 m | Grand Total |
|---------------------------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|-------------|
| *Culex quinquefasciatus*  | 10      | 14      | 56      | 2       | 6       | 17      | 36      | 46      | 10      | 37      | 30      | 264     |
| *Cx sitiens*              | -       | -       | -       | -       | -       | -       | -       | -       | -       | -       | -       | -       | 6          |
| *Cx pseudovishnui*        | 29      | 44      | 81      | 10      | 28      | 41      | 12      | 22      | 49      | 7       | 6       | 27      | 356        |
| *Cx vishnui*              | -       | -       | -       | -       | -       | -       | -       | -       | -       | -       | -       | -       | 6          |
| *Cx mimulus*              | 3       | 2       | 4       | -       | 2       | -       | 2       | 2       | -       | -       | -       | -       | 6          |
| *Cx nigropunctatus*       | 21      | 29      | 25      | 38      | 14      | 18      | 34      | 52      | 33      | 62      | 76      | 402     |
| *Cx (Culiciomyia) bailyi* | 2       | -       | -       | 3       | -       | -       | -       | -       | -       | -       | -       | -       | 5          |
| *Cx (Lutzia) fuscanus*    | -       | -       | -       | -       | -       | -       | -       | -       | -       | 2       | -       | -       | 2          |
| *Orthopodomyia flavithorax* | 23     | 14      | 44      | -       | 7       | 27      | -       | -       | -       | -       | -       | -       | 115        |
| *Or. anopheloides*        | 3       | -       | 4       | -       | 3       | -       | -       | -       | -       | -       | -       | -       | 10         |
| *Coquilletidia crassipes* | -       | -       | 1       | -       | -       | -       | -       | 2       | 3       | -       | -       | -       | 6          |
| *Mansonia annulifera*     | -       | -       | -       | -       | -       | 1       | -       | -       | -       | -       | -       | -       | 3          |
| *Mimomyia chamberlaini*   | -       | -       | -       | -       | -       | -       | -       | -       | -       | 1       | -       | -       | 1          |
| *Aedes albopictus*        | -       | -       | -       | 3       | 2       | -       | 1       | 4       | -       | -       | 2       | 2       | 12         |
| *Armigeres subalbatus*    | -       | -       | -       | 3       | 2       | -       | 1       | 4       | -       | -       | 2       | 2       | 14         |

Grand Total               | 91      | 101     | 219     | 17      | 77      | 93      | 48      | 104     | 163     | 54      | 109     | 136     | 1212       |

*HAN: Hanthana Forest Reserve, HAL: Halgolla Forest Reserve, UOP: Peradeniya University Park, GAM: Gampola.*

Figure 3: The diversity of ornithophilic mosquitoes at three different height levels of the four study sites. BBT: Bird-baited Trap, CT: Control trap, *Ae. al.: Aedes albopictus, Ar. su.: Armigeres subalbatus, Co. cr.: Coquilletidia crassipes, Cx ba.: Cx bailyi, Cx fa.: Cx fuscanus, Cx mi.: Cx mimulus, Cx ni.: Cx nigropunctatus, Cx ps.: Cx pseudovishnui, Cx qu.:Culex quinquefasciatus, Cx si.:Cx sitiens, Cx vi.: Cx vishnui, Ma. an.: Mansonia annulifera, Mi. ch.: Mimomyia chamberlaini, Or. an.: Or. anopheloides, Or. fl.: Orthopodomyia flavithorax.*
Overall, *Cx pseudovishnui* is the only species common to all height levels of selected forests (Figure 3). In addition, *Cx quinquefasciatus* and *Cx nigropunctatus* were also common to all height levels of all sites except for the 1 m height at HAL (Figure 3). Further, *Oc. flavithorax* was reported only in all three heights at HAN, and *Ae. albopictus* was found in all three height levels at GAM. *Culex vishnui* was reported only at 6 m height levels at both HAN and UOP (Figure 3). However, the majority of the ornithophilic *Culex* mosquito species didn’t restrict to a particular height level and were dominated in the tested three strata of the forest. Preference of ornithophilic mosquitoes to the ground level has been reported in a few previous studies (Derraik et al., 2005; Drummond et al., 2006; Jansen et al., 2009; Diallo et al., 2010). According to those authors, *Aedes punctothoracis*, *Anopheles melas*, *An. squamosus*, *Cx annulirostris*, *Cx decens*, *Cx tritaeniorhynchus*, *Ochlerotatus antipodeus*, *Mansonina africana*, and *Ma. uniformis* were frequently found at the ground level. However, no mosquito species was specifically restricted to the forest floor or the understory vegetation during the current study.

Margalef’s Index (d) and Pielou’s Evenness (J’) were higher for the 6 m height level than for the 1 m and 3 m heights in all four study sites (Table 2). However, the diversity of the mosquito fauna in three different height levels was not significantly different according to the calculated diversity indices for all four habitats (p > 0.05) suggesting a non-restricted distribution of ornithophilic mosquito species.

The abundance of ornithophilic mosquitoes

In all study sites, the highest numbers of ornithophilic mosquitoes were collected from the canopy height [HAN (219; 53.3%), and HAL (93; 49.5%), GAM (136; 45.5%), UOP (163; 51.7%)] (Table 1). There was a significant difference in abundance of ornithophilic mosquitoes among the three selected height levels of each study sites [HAN (df = 14, F = 3.26, p = 0.044), HAL (df = 14, F = 10.70, p = 0.002), UOP (df = 14, F = 8.62, p = 0.005), and GAM (df = 14, F = 8.25, p = 0.006)]. Many bird species including communal roosting birds are known to perch on trees at night than in ground-level habitats (Beauchamp, 1999; Janousek et al., 2014). This roosting behaviour may probably have influenced the spatial distribution of ornithophilic mosquitoes providing opportunities to take blood from roosting birds at night when defensive movements of birds are at the minimum level (Janousek et al., 2014; Chathuranga et al., 2018). According to our results, the diversity and the abundance of ornithophilic mosquitoes are relatively high at the canopy level supporting this hypothesis. Previous studies have also shown the high preference of ornithophilic mosquitoes for canopy level (Anderson et al., 2004; Diallo et al., 2010; Swanson and Adler, 2010; Tantely et al., 2019). In those studies, *Aedes kochi*, *Cx astelai*, *Cx pervigilans*, *Cx neavei*, and *Cx weschei* species have been frequently found at the canopy level (Derraik et al., 2005; Drummond et al., 2006; Jansen et al., 2009; Diallo et al., 2010). Our results also reveal a rich mosquito community at the canopy level and we found that bird biting *Cx sitiens*, *Cx fuscanus*, and *Mimomyia chamberlaini* are mainly limited to the canopy level. Whether these mosquitoes breed in breeding sites such as tree holes situated at a higher level in the forest restricting their life cycles to be operative at levels above the grounds or whether they breed at ground level and only the adult life is shifted to the canopy level, would be an interesting research question to answer.

Figure 4 shows the Bray-Curtis similarity matrix for the selected habitat types based on the abundance and diversity of ornithophilic mosquitoes. Interestingly, the similarity matrix clustered into two different groups.

---

Table 2: Diversity indices of ornithophilic mosquitoes at selected study sites and height levels.

| Habitat                  | Height level | Total Species (S) | Margalef’s Index* (d) | Pielou’s Evenness* (J’) |
|--------------------------|--------------|-------------------|-----------------------|------------------------|
| Hanthana Forest Reserve  | 1 m          | 7                 | 1.33                  | 0.82                   |
| (HAN)                    | 3 m          | 4                 | 0.65                  | 0.91                   |
|                          | 6 m          | 10                | 1.67                  | 0.67                   |
| Halgolla Forest Reserve  | 1 m          | 3                 | 0.71                  | 0.87                   |
| (HAL)                    | 3 m          | 5                 | 0.92                  | 0.71                   |
|                          | 6 m          | 6                 | 1.11                  | 0.76                   |
| Peradeniya University Park (UOP) | 1 m          | 4                 | 0.78                  | 0.84                   |
|                           | 3 m          | 7                 | 1.29                  | 0.75                   |
|                           | 6 m          | 8                 | 1.37                  | 0.70                   |
| Gampola (GAM)            | 1 m          | 5                 | 0.81                  | 0.69                   |
|                           | 3 m          | 5                 | 0.85                  | 0.67                   |
|                           | 6 m          | 5                 | 1.00                  | 0.61                   |

*Values are not significantly different between different height levels for all habitat types.
More specialized feeding ornithophilic mosquitoes were present in Hanthana and Halgolla forest reserves which are secondary natural forests while generalized feeding mosquitoes were common in Gampola and Peradeniya university park forested areas which were patchy forests located close to human settlements. For instance, *Cx. quinquefasciatus* is a well-known generalized feeder that shows a wider host range including humans and birds (Janssen *et al.*, 2015), and is reported commonly at all height levels of forested areas in Gampola and Peradeniya university park. However, *Cx pseudovishnui*, a well-known forest-dwelling species that have a specific preference for bird blood (Miyagi and Toma, 1980), was frequently found in Hanthana and Halgolla forest reserves.

Two clusters resulting from Bray-Curtis clustering may indicate that the composition of ornithophilic mosquitoes varied due to the availability of breeding places, easy access to their preferred hosts, vegetation cover, microclimatic conditions, as well as potential impacts of human activities. The highest similarity (89.3%) was shown between the HAN - 1 m and HAL - 6 m height levels. In addition, there was an 88.8% similarity between 3 m and 6 m height levels of GAM. However, pairwise clustering in the same height levels was observed only for 1 m height of UOP and GAM as well as 3 m height level of HAN and HAL.

Amerasinghe and Alagoda (1984), and Jayathilake *et al.* (2015), have reported the spatial distribution of anthropophilic, *Ae. aegypti*, *Ae. albopictus*, and *Ar. subalbatus* mosquitoes in Sri Lanka. To our knowledge, the present study is the first report to show the spatial distribution and niche occupancy of ornithophilic mosquitoes in Sri Lanka. Mosquito-bird interactions at different strata of forest ecosystems are crucial in understanding the transmission of blood-borne parasites from birds to mosquitoes and vice versa. Especially, the mosquitoes of the genus *Culex* have been recognized as vectors of diverse pathogens that are transmissible to humans and other wildlife. For instance, *Cx. pseudovishnui*, a common ornithophilic mosquito has been reported as a major vector for West Nile and Japanese encephalitis viruses (Peiris *et al.*, 1994; Mishra *et al.*, 2001). *Culex quinquefasciatus* also prefers bird hosts and is known to transmit lymphatic filariasis, avian malaria, St. Louis encephalitis, and western equine encephalitis (Kay *et al.*, 1985). Thus, our study opens up many research questions related to enzootic and epizootic cycles of blood pathogens of avifauna in Sri Lanka.

CONCLUSION

We identified the vertical distribution of 15 mosquito species in two secondary natural forests and two forested areas in human settlements in Sri Lanka. The abundance of ornithophilic mosquitoes dominated by the genus *Culex* was significantly different at the forest floor, understory vegetation, and canopy level. However, the diversity of the mosquito fauna in three different height levels was not significantly different. Generalist feeders are frequently found in patchy forests located close the human settlements and more specialized feeders are found in natural forest areas away from human settlements.

ACKNOWLEDGMENT

Funding for this research was provided by the National Research Council, Sri Lanka (NRC Grant No: 16-059), and the University of Peradeniya, Sri Lanka (URG: 2016-51/S). The authors acknowledge Mr. S.L.N. Samarasinghe, Department of Zoology, University of Peradeniya, Sri Lanka for field assistance. The anonymous reviewers are acknowledged for the comments given to improve the manuscript.
DECLARATION OF CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

Amerasinghe, F.P. (1995). Illustrated keys to the genera of mosquitoes (Diptera: Culicidae) in Sri Lanka. Journal of the National Science Council of Sri Lanka 23: 183-211.

Amerasinghe, F.P. and Alagoda, T.S.B. (1984). Mosquito oviposition in bamboo traps, with special reference to Aedes albopictus, Aedes aegypti and Armigeres subalbatus. Insect Science and its Application 5(6): 493-500. DOI: https://doi.org/10.1017/S1742758400004926.

Anderson, J.F., Andreadis, T.G., Main, A.G. and Kline, D.L. (2004). Prevalence of West Nile virus in tree canopy-inhabiting Culex pipiens and associated mosquitoes. American Journal of Tropical Medicine and Hygiene 71: 112–119. DOI: https://doi.org/10.4269/ajtmh.2004.71.112.

Barraud, P.J. (1934). The evolution of communal roosting in birds: origin and secondary losses. Behavioral Ecology 10(6): 675–687. DOI: https://doi.org/10.1093/beheco/10.6.675.

Braack, L.E.O., Coetzee, M., Hunt, R.H., Biggs, H., Cornel, A. and Gericke, A. (1994). Biting pattern and host-seeking behavior of Anopheles arabiensis (Diptera: Culicidae) in Northeastern South Africa. Journal of Medical Entomology 31: 333-339. DOI: https://doi.org/10.1093/jmedent/31.3.333.

Chathuranga, W.G.D., Karunaratne, S.H.P., Fernando, B.R. and De Silva, W.A.P.P. (2018). Diversity, distribution, abundance, and feeding pattern of tropical Ornithophilic mosquitoes. Journal of Vector Ecology 43: 158-167. DOI: http://dx.doi.org/10.1111/jvec.12295.

Clarke, K.R. and Gorley, R.N. (2015). PRIMER v7: User Manual/Tutorial. PRIMER-E, Plymouth, Pp. 1-296.

Diallo, D., Ba, Y., Dia, I., Sall, A.A. and Diallo, M. (2010). Evaluation of the efficiency of bird-baited traps for sampling potential West Nile Fever mosquito vectors (Diptera: Culicidae) in Senegal. Parasite 17: 143-147. DOI: http://dx.doi.org/10.1051/parasite/2010172143.

Derraij, J.G.B., Snell, A.E. and Slaney, D. (2005). Vertical distribution of adult mosquitoes in native forest in Auckland, New Zealand. Journal of Vector Ecology 30(2): 334-336.

Drummond, C., Drobnacl, J., Backenson, P.B., Ebel, G.D. and Kramer, L.D. (2006). Impact of Trap Elevation on Estimates of Abundance, Parity Rates, and Body Size of Culex pipiens and Culex restuans (Diptera: Culicidae). Journal of Medical Entomology 43(2): 177-184. DOI: https://doi.org/10.1603/0022-2585(2006)043[0177:ITEO]2.0.CO;2.

Glaser, F. (2006). Biogeography, diversity, and vertical distribution of ants (Hymenoptera: Formicidae) in Vorarlberg, Austria. Myrmecologische Nachrichten 8: 263-270.

Hayes, J.P. and Gruver, J.C. (2000). Vertical stratification of bat activity in an old-growth forest in western Washington. Northwest Science 74(2): 102–108.

Ishtiaq, F., Guillaumot, L., Clegg, S.M., Phillimore, A.B., Black, R.A., Owens, I.P.F., Bundy, N.I. and Sheldon, B.C. (2008). Avian haematozoan parasites and their associations with mosquitoes across the Southwest Pacific Islands. Molecular Ecology 17: 4545–4555. DOI: https://doi.org/10.1111/j.1365-294X.2008.03935.x.

Janousek, W.M., Marra, P.P. and Kilpatrick, A.M. (2014). Avian roosting behavior influences vector-host interactions for West Nile virus hosts. Parasites and Vectors 7: 399. DOI: https://doi.org/10.1186/1756-3305-7-399.

Jansen, C.C., Zborowski, P., Ritchie, S.A. and van den Hurk, A.F. (2009). Efficacy of bird-baited traps placed at different heights for collecting ornithophilic mosquitoes in eastern Queensland, Australia. Australian Journal of Entomology 48: 53-59. DOI: https://doi.org/10.1111/j.1440-6055.2008.00671.x.

Janssen, N., Fernandez-Salas, I., Diaz Gonzalez, E.E., Gaytan-Burns, A., Medina-de la G., Carlos, E., Sanchez-Casas, R.M., Börstler, J., Cadar, D., Schmidt-Chanasit, J. and Jöst, H. (2015). Mammalophilic feeding behaviour of Culex quinquefasciatus mosquitoes collected in the cities of Chetumal and Cancun, Yucatan Peninsula, Mexico. Tropical Medicine and International Health 20: 1488-1491. DOI: https://doi.org/10.1111/tmi.12587.

Jayathilake, T.A.H.D.G., Wickramasinghe, M.B. and de Silva, B.G.D.N.K. (2015). Oviposition and vertical dispersal of Aedes mosquitoes in multiple-story buildings in Colombo district, Sri Lanka. Journal of Vector-Borne Diseases 52: 245-251.

Jayson, E.A. and Mathew, D.N. (2003). Vertical Stratification and its Relation to Foliage in Tropical Forest Birds in Western Ghats (India). Acta Ornithologica 38(2): 111-116. DOI: https://doi.org/10.3161/068.038.0207.

Kay, B.H., Boreham, P.F. and Fanning, I.D. (1985). Host-feeding patterns of Culex annulirostris and other mosquitoes (Diptera: Culicidae) at Charleville, southwestern Queensland, Australia. Journal of Medical Entomology 22: 529–535. DOI: https://doi.org/10.1093/jmedent/22.5.529.

Lequime, S. and Lambrechts, L. (2014). Vertical transmission of arboviruses in mosquitoes: A historical perspective. Infection, Genetics and Evolution 28: 681-690. DOI: http://dx.doi.org/10.1016/j.meegde.2014.07.025.

Livak, K.J. (1984). Organization and mapping of a sequence on the Drosophila melanogaster X and Y chromosomes that is transcribed during spermatogenesis. Journal of Genetics 107: 611–634.

Meister, T., Lussy, H., Bakonyi, T., Šikutová, S., Rudolf, I., Vogl, W. and Weissenböck, H. (2008). Serological evidence of continuing high Usutu virus (Flaviviridae) activity and establishment of herd immunity in wild birds in Austria. Veterinary Microbiology 127: 237-248. DOI: https://doi.org/10.1016/j.vetmic.2007.08.023.
Meneses, A.R., Querino, R.B., Oliveira, C.M., Maia, A.H.N. and Silva, P.R.R. (2016). Seasonal and Vertical Distribution of Dalbatus maida (Hemiptera: Cicadellidae) in Brazilian Corn Fields. *Florida Entomologist* 99(4): 750-754. DOI: https://doi.org/10.1653/024.099.0428.

Mishra, A.C., Jadi, R.S., Paramasivan, R. and Mourya, D.T. (2001). Antigen distribution from Germany. *Culex tritaeniorhynchus, Culex vishnui* and *Culex pseudovishnui* mosquitoes. *Journal of Communication Disorders* 33: 174–179.

Miyagi, I. and Toma, T. (1980). Studies on the mosquitoes in Yaeyama Islands, Japan. 5. Notes on the mosquitoes collected in forest areas of Irinomejima. *Medical Entomology and Zoology* 31(2): 81-91. DOI: https://doi.org/10.7601/mez.31.81.

Molaeli, G., Cummings, R.F., Su, T., Armstrong, P.M., Williams, G.A., Cheng, M.L. and Andreadis, T.G. (2010). Vector-host interactions governing epidemiology of West Nile virus in Southern California. *American Journal of Tropical Medicine and Hygiene* 83:1269-1282. DOI: https://doi.org/10.4269/ajtmh.2010.10-0392.

Nolan, D.V., Carpenter, S., Barber, J., Mellor, P.S., Dallas, J.F., Mordue, A.J.L. and Piertney, S.B. (2007). Rapid diagnostic PCR assays for members of the *Culicoides obsoletus* and *Culicoides pulicaris* species complexes, implicated vectors of bluetongue virus in Europe. *Veterinary Microbiology* 124: 82–94. DOI: https://doi.org/10.1016/j.vetmic.2007.03.019.

Peiris, J.S.M., Amerasinghe, P.H., Amerasinghe, F.P., Calisher, C.H., Perera, L.P., Arunagiri, C.K., Munasingha, N.B. and Karunaratne, S.H.P.P. (1994). Virus isolation from mosquitoes collected in Sri Lanka. *American Journal of Tropical Medicine and Hygiene* 51(2): 154-161. DOI: https://doi.org/10.4269/ajtmh.1994.51.154.

Pekar, S. (2005). Horizontal and Vertical Distribution of Spiders (Araneae) in Sunflowers. *Journal of Arachnology* 33(2): 197-204. DOI: https://doi.org/10.1636/04-54.1.

Rogosi, E., Bego, F., Papa, A., Mersini, K. and Bino, S. (2013). Distribution and ecology of small mammals in Albania. *International Journal of Environmental Health Research* 23(3): 258-268. DOI: https://doi.org/10.1080/09603123.2012.717917.

Rohner, C., Krebs, C.J., Hunter, D.B. and Currie, D.C. (2000). Roost site selection of great horned owls in relation to blackfly activity: an anti-parasite behaviour?. *Condor* 102(4): 950–955. DOI: https://doi.org/10.1650/0010-5422(2000)102[0950:RSSOGH]2.0.CO;2.

Simon, C., Frati, F., Beckenbach, A., Crespi, B., Liu, H. and Flook, P. (1994). Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Annals of the Entomological Society of America* 87(6): 651-701. DOI: https://doi.org/10.1093/aeas/87.6.651.

Subasinghe, K., Sumanapala, A.P. and Weerawardhena, S.R. (2014). The impact of forest conversion on bird communities in the northern flank of the Knuckles Mountain Forest Range, Sri Lanka. *Journal of Asia-Pacific Biodiversity* 7(4): 367e373. DOI: https://doi.org/10.1016/j.japb.2014.07.004.

Swanson, D.A. and Adler, P.H. (2010). Vertical distribution of haematophagous Diptera in temperate forests of the southeastern U.S.A. *Medical and Veterinary Entomology* 24(2): 182–188. DOI: https://doi.org/10.1111/j.1365-2915.2010.00862.x.

Tantely, L.M., Randrianambinintsoa, F.J., Woog, F., Raharinirina, M.R., Ratsimbazafy, J., Boyer, S. and Girod, R. (2019). Horizontal and vertical distribution of mosquitoes (Diptera: Culicidae) in the rainforest of Maromizaha, Madagascar: implications for pathogen transmission to humans and animals. *Austral Entomology* 58(4): 897-906. DOI: https://doi.org/10.1111/aen.12427.

Ulyshen, M.D. (2011). Arthropod vertical stratification in temperate deciduous forests: Implications for conservation-oriented management. *Forest Ecology and Management* 261(9):1479-1489. DOI: https://doi.org/10.1016/j.foreco.2011.01.033.

Valkiūnas, G. (2005). Avian Malaria Parasites and Other Haemosporidian. CRC Press, Boca Raton, FL, USA, 932.

Weeraratne, T.C., Surendran, S.N., Walton, C. and Karunarathne, SH.P.P. (2018). Genetic diversity and population structure of malaria vector mosquitoes *Anopheles subpictus, Anopheles peditaeniatus,* and *Anopheles vagus* in five districts of Sri Lanka. *Malaria Journal* 17: 271. DOI: https://doi.org/10.1186/s12936-018-2419-x.

Zotzmann, S., Steinbrink, A., Schleich, K., Frantzmann, F., Xoumpholphakdy, C., Spaeth, M., Valiente Moro, C., Mavingui, P. and Klimpel, S. (2017). Bacterial diversity of cosmopolitan *Culex pipiens* and invasive *Aedes japonicus* from Germany. *Parasitology Research* 116(7): 1899-1906. DOI: https://doi.org/10.1007/s00436-017-5466-2.