# THE PMI VECTORLINK PROJECT CAMBODIA ANNUAL ENTOMOLOGY REPORT DECEMBER 2021 – APRIL 2023

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# ACRONYMS

| | |
|---|---|
| **b/c/n** | bites per cow per night |
| **b/p/n** | bites per person per night |
| **CDN** | Cow-Baited Double-Net Trap |
| **CDC** | U.S. Centers for Disease Control and Prevention |
| **CNM** | National Center for Parasitology, Entomology and Malaria Control |
| **CS** | Circumsporozoite Protein |
| **ELISA** | Enzyme-linked Immunosorbent Assay |
| **GIS** | Geographic Information System |
| **GPS** | Global Positioning System |
| **HBR** | Human Biting Rate |
| **HDN** | Human-Baited Double-Net Trap |
| **ITN** | Insecticide-Treated Net |
| **LT** | Light Trap |
| **MIS** | Malaria Information System |
| **OD** | Operational District |
| ***P.*** | *Plasmodium* |
| ***P.f*** | *Plasmodium falciparum* |
| ***P.v*** | *Plasmodium vivax* |
| **PCR** | Polymerase Chain Reaction |
| **PHD** | Provincial Health Department |
| **PMI** | President's Malaria Initiative |
| **PMS** | Provincial Malaria Supervisor |
| **SOP** | Standard Operating Procedure |
| **TOT** | Training of Trainer |
| **WHO** | World Health Organization |

# EXECUTIVE SUMMARY

Longitudinal and case-based entomological monitoring, insecticide resistance monitoring, capacity strengthening, and development of the data management entomology module in the current malaria information system of the National Center for Parasitology, Entomology and Malaria Control (CNM) were the main activities VectorLink Cambodia performed in collaboration with the CNM from December 2021 to April 2023. Bimonthly entomological monitoring was conducted for five months, from December 2021 to August 2022, in four sites comprising two annex village sites and two forest fringe sites in Mondulkiri and Stung Treng provinces. Three mosquito trapping methods, outdoor cow-baited double-net traps (CDNs), outdoor human-baited double-net traps (HDNs), and indoor U.S. Centers for Disease Control and Prevention (CDC) light traps, were used in the annex village sites, while only outdoor HDNs were used in the forest fringe sites. In addition, mosquito collections for insecticide resistance monitoring were conducted bimonthly at the four sites.

A total of 12,805 *Anopheles* species mosquitoes were collected across all provinces and collection methods. Thirty-two *Anopheles* species were found in Mondulkiri and Stung Treng, two malaria-endemic provinces in Cambodia. Among the three primary malaria vectors, *An. minimus* sensu lato was most abundant in the village site (12.68%, n=850/6,705) of Mondulkiri, while *An. dirus* s.l. was most abundant in the forest fringe site (50.99%, n=359/704). In Stung Treng, *An. dirus* s.l. was most abundant in both the village sites (10.78% n=468/4,343) and forest fringe sites (78.06% n=822/1,053). In annex villages, CDNs caught the highest numbers of *An. minimus* s.l. and *An. maculatus* s.l. mosquitoes while HDNs collected the highest number of *An. dirus* s.l. This establishes use of these collection methods for measuring the presence or absence of primary vectors in these two endemic provinces.

Similar to the previous year, the highest mean human biting rates estimated from HDNs among the three primary vectors were observed in *An. dirus* s.l. in Stung Treng; they were lower in Mondulkiri. Conversely, the human biting rates of *An. minimus* s.l. and *An. maculatus* s.l. were higher in Mondulkiri than in Stung Treng. The enzyme-linked immunosorbent assay (ELISA) results of bimonthly collections indicated that *An. minimus* s.s. was found positive for *Plasmodium falciparum* and *An. dirus* s.s. was positive for both *P. falciparum* and *P. vivax* infection. The continued exposure to these primary malaria vectors explains the residual malaria transmission that persists in the two provinces.

The three primary vectors were found biting actively in the forest in both provinces from 18:00 and throughout the night up to 06:00 hours. This presents potential risk to forest goers who might be working at night and unprotected from mosquito bites and underlines the need for personal protection tools such as topical repellents as a supplementary tool to insecticide-treated nets. Outdoor peak biting time varied between 18:00 and 23:00, coinciding with the sleeping pattern of people in the villages, where approximately 11% in Stung Treng and 35% in Mondulkiri remained outdoors and exposed at those times. Similar biting trends were observed in the previous collection period. These results highlight the need for vector control tools that can be used outdoors, such as topical repellents and hammock nets.

Out of the six local *Plasmodium* (*P.*) *falciparum* foci cases reported and responded to by conducting entomological surveillance, four were reported in Mondulkiri and two in Stung Treng. Primary vectors were detected at each focus confirming receptivity for malaria transmission. During each focus investigation, entomological data contributed to the receptivity score, which also includes malaria case management data. These data are used to determine the best course of action with the objective of interrupting onward transmission.

All (231) morphologically identified primary vectors (*An. dirus* s.l. n=199; *An. maculatus* s.l. n=27; *An. minimus* s.l. n=5) collected during the six foci investigations (May 2022–March 2023) were analyzed using enzyme-linked immunosorbent assay (ELISA) to determine the *P. falciparum* and *P. vivax* infections and sibling species identification. The results indicated one *An. dirus* s.l., from Royang village in Mondulkiri Province, was positive for *P. vivax* 210. The molecular identification confirmed 82.91% (165/199) were identified as *An. dirus* s.s, out of these 34 numbers of specimens did not amplify. From *An. minimus* s.l. 40.00% (2/5) were identified as *An. minimus* s.s. among these three specimens did not amplify. For *An. maculatus* s.s., (0/27), zero percent was the molecular confirmation. Out of the 27 morphologically identified *An. maculatus* s.l. molecular identification indicated 48.14% (13/27) *An. sawadwongporni,* 40.74% (11/27) *An. rampae* (form K) and three specimens did not amplify.

A total of 1,993 *Anopheles* vector mosquitoes 59.22% (1,993/3,365) from total *Anopheles* vectors collected during the longitudinal study from December 2021 to August 2022 were analyzed using enzyme-linked immunosorbent assay (ELISA) to determine the *P. falciparum* and *P. vivax* infections of the collected mosquitoes. From Mondulkiri Province, 924 *Anopheles* were tested (*An. dirus* s.l. 303, *An. maculatus* s.l. 266, and *An. minimus* s.l. 355), and two mosquitoes were found harboring *Plasmodium* infection - one each for *P. falciparum* and *P. vivax*. Of the 1,069 primary vectors screened from Stung Treng Province (*An. dirus* s.l. 1,021, *An. maculatus* s.l. 37, and *An. minimus* s.l. 11), one found with *P. falciparum* infection. In Mondulkiri, the sporozoite positivity in the primary vector mosquitoes indicates that malaria transmission persists in the area and the community is at risk of infection indicating the need for sustained use of vector control interventions.

Field-collected *An. dirus* s.l. mosquitoes from forest fringe sites of Mondulkiri were susceptible to deltamethrin 0.05%. Similarly, *An. maculatus* s.l. was found susceptible to alpha-cypermethrin 0.05% and deltamethrin 0.05% from the village site and also susceptible to deltamethrin 0.05% from the forest site. Further, *An. minimus* s.l. from annex village of Mondulkiri showed susceptibility to alpha-cypermethrin 0.05%, permethrin 0.75% and deltamethrin 0.05%. In the Stung Treng province, *An. dirus* s.l. was susceptible to deltamethrin 0.05% in the annex village and permethrin 0.75% and alpha-cypermethrin 0.05% for the forest fringe. *An. maculatus* s.l. from the forest site was found susceptible to deltamethrin.

# I. INTRODUCTION

The World Health Organization (WHO) reported a global reduction of 74% in malaria cases and 76% reduction in malaria deaths in 2021, from 2000. Further, the Greater Mekong Subregion countries, including Cambodia, Laos, Vietnam, Myanmar, Thailand, and China (Yunnan Province), reported a 76.5% decrease in overall malaria cases and a 94.1% decline in indigenous malaria cases between the two years (WHO 2022). Though Cambodia's malaria burden has reduced significantly since the last decade, approximately 9.3 million (53%) people from 55 high-endemic operational districts (ODs) are living at risk. The intensity of transmission has been observed to vary significantly across different ecological zones. with transmission highest in the forested or forest fringe areas in the northeastern part of the country. Malaria cases in Cambodia are primarily caused by *Plasmodium* (*P.*) *falciparum, P. vivax*, or a mix of both species, while the main malaria vectors are *Anopheles dirus* s.l.*, An. minimus* s.l., and *An. maculatus* s.l. Malaria transmission occurs primarily during the rainy season in July and November. *An. dirus* s.l. is found in forested mountains and foothills, cultivated forests, and rubber plantations, whereas *An. minimus* s.l. is found outside the forests or in areas that have been cleared of forest. *An. maculatus* s.l. is found in hilly or mountainous areas and breeds in or near permanent or semi-permanent bodies of clean water, such as streams or rivers. To a small extent, members of other species groups, such as *An. hyrcanus* and *An. barbirostris,* also contribute to malaria transmission.

In support of the Government of Cambodia's malaria elimination goal, the U.S. President's Malaria Initiative (PMI) VectorLink Project has been conducting entomological monitoring activities in Cambodia, focusing on malaria higher-burden areas, since 2019. The primary goal of the project is to support entomological surveillance in high-burden districts including longitudinal surveillance and complementary case-based entomological surveillance in response to *P. falciparum* L1 cases which are defined as an indigenous malaria case for an individual who has spent every night within their current residence in the last two weeks (i.e., not travelled) reported in endemic provinces. The data are used to guide and inform vector control decisions. The PMI VectorLink project also conducts annual insecticide resistance monitoring of the pyrethroids used on insecticide-treated nets (ITNs). In addition, PMI VectorLink aims to strengthen the capacity of the National Center for Parasitology, Entomology and Malaria Control (CNM), provincial health department (PHDs), and ODs to enable the decentralization of entomological surveillance activities to the provincial and district levels.

The specific objectives of VectorLink Cambodia are as follows:

- To characterize *Anopheles* species composition, density, seasonality, and biting behavior
- To measure malaria vector occurrence
- To determine the insecticide susceptibility of the main malaria vectors to pyrethroid insecticides
- Molecular analysis of *Anopheles* mosquito samples, generating sporozoite rates using circumsporozoite protein (CS) enzyme-linked immunosorbent assays (ELISAs) for *P. falciparum* and *P. vivax*, and determining species identification by polymerase chain reaction (PCR)
- Capacity strengthening of entomological manpower involved in the malaria control program.

Entomology capacity strengthening on *Anopheles* mosquito morphological identification was conducted jointly with CNM through four training programs for 71 participants from nine PHDs: Mondulkiri, Stung Treng, Kampot, Ratanak Kiri, Pursat, Koh Kong, Kratie, Kampong Speu, and Preah Vihear.

Entomological monitoring and insecticide resistance monitoring was conducted from December 2021 to April 2023 in two high malaria-endemic provinces, Stung Treng and Mondulkiri (Figure 1), which had an Annual Parasite Index of 3.2 (2021) and 5.7(2022), and 6.1 (2021) and 5.3 (2022), respectively. VectorLink

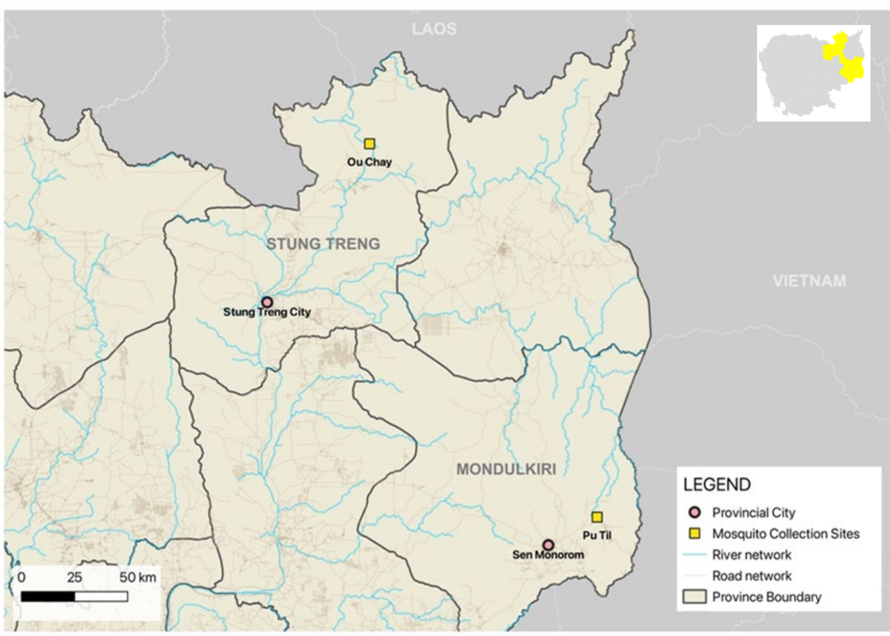
Cambodia, in partnership with CNM and the two PHDs and their ODs, conducted bi-monthly longitudinal entomological monitoring in annex villages and a forest sentinel sites in the two provinces. All field collected mosquito samples were analyzed at the University of Puthisastra. The National Institute of Health established a capacity for CS ELISA and began sample processing on May 16, 2022. Molecular lab training for VectorLink Cambodia was completed in August 2022. For establishing project laboratory space and processing samples without interruption, in January 2023, a memorandum of understanding was signed with the University of Puthisastra. The PCR sample processing for sibling species identification started in May 2023. All resulted in the data shared in this report.

# 2. METHODOLOGY

## 2.1 SAMPLING SITES AND COLLECTION METHODS

Entomological monitoring was conducted in four sentinel sites in two highly malaria endemic provinces, Stung Treng and Mondulkiri. The sentinel sites consist of two annex villages and two forest fringe sites (Figure 1). The annex villages are informal settlements, often geographically isolated and created due to population growth or movement from the main villages. The annex villages include Pu Til in Pech Chreada District of Mondulkiri Province and Ou Chay in Siem Pang District of Stung Treng Province. The forest fringe sites are located approximately 1.5–2 km away from the villages. The Pu Til sites are characterized by hilly forested areas, while Ou Chay sites are lowland forests intersecting with rivers and streams. Villagers often enter the forest for farming, cutting trees, hunting, and other activities. The main occupation of the villagers is agriculture, such as rice farming in Ou Chay and cassava, rubber, and pepper farming in Pu Til. The sentinel sites have a tropical climate, with temperatures ranging from 17°C to 38°C (Matsumoto 1997; Tsuji moto et al. 2018). The rainy season is from May to October, followed by the dry season from November to April. In collaboration with CNM, VectorLink Cambodia initiated entomological surveillance in response to *P. falciparum* L1 cases reported in Mondulkiri and Stung Treng provinces in May, July, and September 2022 and in March 2023, with the objective of determining the presence and absence of malaria vectors as well as their indoor and outdoor biting behavior.

**Figure 1. Sentinel Sites for Entomological Monitoring: Ou Chay Sites in Siem Pang District, Stung Treng Province and Pu Til Sites in Pech Chreada District, Mondulkiri Province**




The mosquito sampling methods used during longitudinal monitoring included: outdoor cow-baited double-net traps (CDNs), human-baited double-net traps (HDNs), and indoor CDC light traps (CDC-LTs) (Table 1).

**Table 1. Adult Mosquito Collection Methods Used for Longitudinal Entomological Monitoring**

| Collection Method | Time | Frequency and Site | Sample | Indicators |
|---|---|---|---|---|
| CDN | Hourly collections 18:00 to 6:00 | Bimonthly for annex village sites and foci investigations | Three traps/month/site | Outdoor cattle biting rate: number per trap per hour, Sporozoite rate |
| HDN | Hourly collections 18:00 to 6:00 | Bimonthly for all sites and foci investigations | Three traps/month/site | Outdoor human biting rate: number per trap per hour, Sporozoite rate |
| Indoor CDC-LT | 18:00 to 6:00 | Bimonthly for annex village sites and foci investigations | Six traps/month/site | Number per trap, Sporozoite rate |

#### 2.1.1 COW-BAITED DOUBLE NET TRAP

CDNs were used outdoors for longitudinal surveillance, insecticide resistance monitoring, and case-based entomological surveillance. They were set up in the annex village sites and foci reported villages only, because it was difficult to obtain cows to use in the forest sites. The cow was kept inside the double net from sunset to dawn. Collections were carried out using glass tubes to catch the mosquitoes trapped inside the outer net. Hourly collections were made according to PMI VectorLink Standard Operating Procedure for CDN traps. From December 2021, after each hour of manual collection of mosquitoes, a Prokopack aspirator was used to catch any remaining mosquitoes inside the trap to check the efficiency of the collectors using the glass tubes.

#### 2.1.2 HUMAN-BAITED DOUBLE NET TRAP

HDN collections were conducted outdoors starting in October 2020. They were set up in the annex village sites and in the forest sites. A human volunteer slept inside the double net from sunset to dawn. Collections were carried out using glass tubes to catch the mosquitoes trapped inside the outer net. Hourly collections were made according to PMI VectorLink SOP 02/01. From June 2021, after each hour of manual collection of mosquitoes, a Prokopack aspirator was used to catch the remaining mosquitoes inside the trap.

#### 2.1.3 CDC LIGHT TRAPS

Indoor CDC-LT collections were conducted starting in December 2021. These traps were set up in four annex village houses' bedrooms next to a volunteer who would sleep under an existing insecticide treated net (ITN). Mosquitoes trapped inside the collection cup were collected the following morning, in accordance with PMI VectorLink SOP 03/01.

### 2.2 CASE-BASED ENTOMOLOGICAL INVESTIGATION

In collaboration with CNM, VectorLink Cambodia conducted entomological surveillance in response to *P. falciparum* L1 cases reported in Mondulkiri and Stung Treng provinces in July and September 2022, with the objective of determining the presence and absence of malaria vectors and sporozoite rate. In the first 15 days of a foci investigation month, VectorLink Cambodia responded to the first two *P. falciparum* L1 cases reported in Stung Treng and Mondulkiri provinces. Cases are reported to VectorLink Cambodia via an SMS monitoring system, but to mitigate against any delays or errors with that system, VectorLink Cambodia also called the provincial malaria supervisor (PMS) in both districts twice a week. The PMS receives reports from village malaria workers of any new cases. The village malaria workers and mobile malaria workers report verbally to the PMS, while health centers report to the PMS by Telegram. The planning of the investigation visit is prepared by the stakeholders (CNM, PHD, OD, and partner organizations in Mondulkiri and Stung Treng) and community leaders. The investigations generally last four consecutive days (three nights). The foci investigation is initiated from the OD level, led by the OD malaria supervisor and a technician. The PMS also takes part in the foci investigation, assisted, if necessary, by staff from the closest health center and active village malaria workers, if in place. The VectorLink team coordinates with this focus investigation team and provides technical assistance in the entomological aspects of the investigation. The OD-led investigation team identifies the geographical coordinates of the L1 index case and draws a sketch of the surrounding area containing features such as roads, rivers, water bodies, farms, and forests. Whenever possible, the GPS coordinates of those geographical features are plotted onto a map using GIS software. VectorLink Cambodia uses GIS software to create and overlay a 1km radius around the residence of the L1 case, to demarcate the area in which the VectorLink-supported entomological survey will be conducted. A suitable building or room is identified where microscopes can be set up, CDC-LT batteries can be charged, mosquito samples (adults and larvae) can be sorted and packed, and equipment can be stored when not in use. The room should have a reliable supply of electricity and be secure. Mosquito sampling is conducted in the residence of the L1 cases and within a 1km radius.

During foci investigations in 2022, the CDC-LTs were placed in five houses including the L1 case house, while one HDN trap was placed 50m from the L1 case house (Table 2). Mosquito sampling was conducted for three consecutive nights in a month during longitudinal surveillance as well as during each foci investigation. Larval sampling was conducted in July 2022 within a 1km radius of the L1 case house but was discontinued after July due to insecurity at the sites.

**Table 2. Sites and Methods for Case-Based Entomological Surveillance**

| Collection Method | Time | Foci Response Coverage | Trapping Frequency | Sample |
|---|---|---|---|---|
| HDN trap | Hourly collections, 18:00–06:00 | Mondulkiri and Stung Treng provinces | Three collection nights at L1 cases reported village | One trap per site (3 trap nights/foci site) |
| CDN trap | Hourly collections, 18:00–06:00 | Mondulkiri and Stung Treng provinces | Three collection nights at L1 cases reported village | One trap per site (3 trap nights/foci site) |
| Indoor CDC-LT | Hourly collections, 18:00–06:00 | Mondulkiri and Stung Treng provinces | Three collection nights at L1 cases reported village | Five houses with one CDC-LT per house (15 trap nights/foci site) |

## 2.3 Human Behavioral Survey to Understand Gaps in Protection

This survey was conducted in collaboration with CNM and aims to measure and characterize human behavior as it relates to the biting behavior of malaria vectors. Information on how humans overlap with vectors in time and space was collected via household questionnaires. Mosquito behavior and human behavior data was used to estimate the risk of exposure to malaria vectors and determine the gaps in protection for users of ITNs and hammock nets. The specific objectives of the study were to quantify and characterize human behavior occurring both indoors and outdoors and to estimate the risk of human exposure to malaria vectors outdoors and at different times of the evening and night. The first round of the survey was conducted during the dry season in March 2023 in the annex villages of Pu Til in Mondulkiri Province, and Ou Chay in Stung Treng Province. Upon signing the written informed consent, a questionnaire (Annex 1) was administered to the head of household or representative by trained interviewers. The interviews took approximately 15–20 minutes each. Thirty-one households were surveyed and information on 103 people was gathered in Pu Til village; in Ou Chay, 15 households were interviewed, and information was gathered on 79 people.

## 2.4 Morphological Identification of *Anopheles* Mosquitoes and Storage

All *Anopheles* mosquitoes collected using the three methods were identified morphologically and cross-checked by the VectorLink Cambodia entomology supervisory team in the field. Female *Anopheles* mosquitoes collected by CDC-LTs were classified according to the four abdominal stages (unfed, fed, gravid, and half-gravid). All *Anopheles* were initially preserved in 1.5 mL Eppendorf tubes with silica gel and transferred later to a freezer for preservation for future molecular laboratory analysis.

## 2.5 Molecular Analysis

Following the establishment of molecular capacity at and partnership with the University of Puthisastra in 2022 and 2023, VectorLink Cambodia began molecularly processing a backlog of samples from longitudinal monitoring and foci investigation collection sites from December 2021 to March 2023. To determine sporozoite rates of primary vectors in collection sites, CS ELISAs were conducted to identify *P. vivax* and *P. falciparum* sporozoites (Wirtz et al. 1992, Burkot et al. 1994). Out of 3365 primary vectors collected 1,993 specimens (59.22%) were tested for both *Plasmodium* sporozoites, including 1,324 *An. dirus* s.l.*,* 303 *An. maculatus* s.l., and 366 *An. minimus* s.l. Following initial screening of *P. falciparum, P. vivax* 247, and *P. vivax* 210, all CS-positive homogenates were boiled and retested for confirmation of true positivity (Durnez et al. 2011). Similarly, all 231 primary vector specimens (100%) collected from six foci investigations were also tested for the same (199 *An. dirus* s.l.*,* 27 *An. maculatus* s.l., and 5 *An. minimus* s.l.).

To establish species composition of the collection sites, all 231 collected specimens (100%) identified morphologically as primary vector species from Mondulkiri and Stung Treng provinces during the period of May 2022 to March 2023 were identified to sibling species level by conventional PCR. The DNA from legs and wings of individual mosquitoes were extracted using QuantaBio Extracta DNA prep for PCR kit (Beverly, MA, USA). Specimens identified as *An. dirus* s.l., *An. maculatus* s.l., and *An. minimus* s.l. were further identified to sibling species using conventional PCR according to Walton et al. 1997, Walton et al. 2007, and Garros et al. 2006, respectively.

## 2.6 Insecticide Resistance Monitoring

Insecticide susceptibility tests were conducted from December 2021 through April 2023 following PMI VectorLink SOP 06/01, based on the World Health Organization (WHO) susceptibility tube test procedure. Adult females of *An. dirus* s.l., *An. maculatus* s.l.*,* and *An. minimus* s.l. collected by the HDN and CDN methods were used.

The tests prioritized pyrethroids of the ITNs used in the annex villages in both provinces. In Mondulkiri Province, the ITNs used in the annex village houses were PermaNet 2.0 (deltamethrin), SafeNet (alpha-cypermethrin), and Yahe (deltamethrin). In Stung Treng Province, the ITNs used in the annex village houses were SafeNet and PermaNet 2.0, with a majority being SafeNet. Thus, the three pyrethroids (alpha-cypermethrin, deltamethrin, and permethrin) were tested against the three selected primary vectors. The exposure to diagnostic dose test papers was 60 minutes, and mortality was recorded 24 hours after exposure.

Interpretation of the results followed WHO guidance, i.e., 98–100% mortality is defined as susceptibility, 90–97% mortality as possible resistance (further investigations needed), and less than 90% mortality as confirmed resistance. To increase the mosquito numbers collected for susceptibility tests, VectorLink Cambodia doubled the number of HDNs in the forest sites from September 2021 through November 2021 and in both annex villages and forest fringe sites in November 2021 in the two provinces.

## 2.7 CAPACITY STRENGTHENING OF ENTOMOLOGICAL MANPOWER INVOLVED IN THE MALARIA CONTROL PROGRAMME

In the context of technical assistance for foci investigations, the strengthening of knowledge and skills needed for *Anopheles* species identification, particularly at the provincial level and other subnational levels, is seen as a priority. Identification of *Anopheles* mosquitoes, particularly primary vectors, to the species level is a vital skill required of the field staff engaged in malaria entomological monitoring, to contribute to a score of 'receptivity' for a given location, and to support a rapid response. Usually, identification of *Anopheles* mosquitoes is done using a stereomicroscope and following a dichotomous key. In Cambodia, there are 53 *Anopheles* species, of which *An. dirus* s.l., *An. maculatus* s.l., and *An. minimus* s.l. are the primary vectors. In collaboration with CNM, it was decided to provide classroom practical and theoretical training on morphological identification of *Anopheles* species of Cambodia to PHD, OD, and health center staff. The trainees are subsequently expected to be fully engaged in the case-based entomological surveillance of foci investigations.

Specific objectives of the training were: (1) to provide basic knowledge of mosquito lifecycle and behavior; (2) to provide theoretical and practical training in morphological identification of *Anopheles* mosquitoes, and specifically the primary vectors, of Cambodia. The theory was provided through a series of Microsoft PowerPoint presentations. The practical sessions initially focused on how to use the microscopes, and subsequently identify prepared pinned mosquito specimens of Culicine and Anopheline samples, including the three primary vectors. To maintain the quality of the training, the facilitation team conducted daily reflection sessions using feedback from participants collected using a daily evaluation form and observations from the training team members themselves. The action points from each day were identified and addressed for the next day of training. Thirty-minute re-cap sessions were conducted on the morning of day two and day three. To evaluate the knowledge and skill of the trainees before and after training, a pre- and post-test was taken. The training covered 12 topics as follows:

- Entomology in Malaria Elimination
- Introduction to *Anopheles* Mosquitoes: Lifecycle, Basic Morphological Identification, and the Difference between Anopheles vs Culex and Aedes
- Introduction to Stereomicroscopy
- Introduction to Adult Mosquito External Morphological Characteristics Important Characteristics for *Anopheles* Morphological Identification
- Introduction to Adult Mosquito External Morphological Characteristics Morphology of Primary Vectors in Cambodia
- Practice Mosquito Identification using Pictorial Keys for Subgenus *Cellia* vs Subgenus *Anopheles*– Series and Species Group: Adult
- Practice Mosquito Identification using Pictorial keys for Subgenus *Cellia*/*Leucophyrus* group
- Practice Mosquito Identification using Pictorial keys for Subgenus *Cellia*/*Funestus* group
- Practice Mosquito Identification using Pictorial keys for Subgenus *Cellia*/*Maculatus* group
- Practice Mosquito Identification using Pictorial keys for Subgenus *Cellia*/*Barbirostris* group
- Mosquito Identification for Receptivity in Malaria Elimination Settings.
- CNM and VectorLink Cambodia jointly conducted four training workshops between November 2022 and March 2023 (Table 3)

**Table 3. Summary of Entomological Training Sessions**

| Session | Location | Date | Provinces Trained |
|---|---|---|---|
| 1 | Pursat Province at PHD Pursat | November 30 to December 2, 2022 | Pursat Province |
| 2 | Kampong Cham at LBN hotel | January 16 to 18, 2023 | Mondulkiri, Kratie, and Kampong Speu provinces |
| 3 | Koh Kong at the meeting hall of the Koh Kong PHD | January 25 to 27, 2023 | Koh Kong and Kampot provinces |
| 4 | Kampot at Sunny hotel | March 20 to 22, 2023 | Stung Treng, Ratanak Kiri, and Preah Vihear provinces. |

The facilitation team included leader trainers - Ms. Mao Sokny (CNM); Dr Matthew Kirby, Dr Sheila Ogoma, Dr Arun Sivan, Mr. Noch Nin, and Mr. Yan Chanly (VectorLink) - to present the course materials and lead the training sessions. Training assistants -Mr. Kang Thavuth, Mr. Ou Theany, and Mr. Sroy Bolin (CNM); Mr. Tat Baura and Ms. Yim Chanmuneneath (VectorLink) - provided hands-on assistance to trainees in the use of microscopes, to distribute the mosquito samples to the trainees for practice of identification throughout the training, as well as to gather participant feedback and organize the pre-training and post-training tests.

A total of 71 participants were trained across the four sessions, of which nine were female. Participants included representatives from the PHD, OD, and health center. The CMEP2 project provided logistical and financial support to the training course and VectorLink Cambodia and CNM administered the training sessions.

To evaluate the knowledge and skill of the trainees before and after training, a nine-question test was used: eight theoretical questions and one question assessing their ability to correctly identify five unlabeled mosquito samples. Each participant had 30 minutes to complete the test, divided approximately into 15 minutes for the theory and 15 minutes with a microscope and the unlabeled samples.

## 2.8 TRAINING OF TRAINERS ON MIS

VectorLink Cambodia has worked together with CNM entomology and malaria information system units to build entomology modules into the existing web-based malaria information system (MIS). VectorLink has outsourced a software development consultant since July 2022 to build new modules allowing for the recording and reporting of mosquito collection data from sentinel site and foci sites, and insecticide resistance data. By December 2022, the software package was ready to be deployed to the MIS production server. Therefore, the training of trainers (TOT) session was conducted for key personnel at the MIS and entomology units, as was training with selected provincial malaria program staff from Mondulkiri and Stung Treng.

The training served specific objectives (1) Introduce entomology data collection methodology and insecticide resistance methodology, (2) Introduce the entomology data collection forms of mosquito collection and insecticide resistance testing - paper-based forms and user interface in the system, (3) Introduce the functionality of the entomology module, and (4) Practice data entry for mosquito collection form and insecticide resistance testing form.

Training on theory was presented using PowerPoint presentations, while practical exercises were conducted directly using the MIS throughout the training. Participants were provided user accounts to perform data entry in the Test Server. A one-day TOT course on the use of entomology modules in the MIS was jointly conducted by the VectorLink Cambodia team, MIS unit chief, and the software consultant on December 30, 2022, in Classy Hotel in Battambang Province.

The training team included:

- Regional Technical Advisor Dr. Sheila Ogoma, who remotely facilitated two topics via Microsoft Teams (with the interpretation of Entomology Technical Lead Mr. Noch Nin).
- VectorLink Chief of Party Mr. Im Chanry, who facilitated the topics of paper-based data collection forms and processes.
- Chief of CNM MIS Unit Mr. Ngor Pengby and Software Consultant Ms. Eng Thyda, who facilitated the topics on how to use the entomology modules in the system for data entry and reporting.

Ten participants (one female) included one MIS unit staff, one entomology unit chief, two entomology technicians, three participants from Stung Treng, and three participants from Mondulkiri. An additional two technicians from VectorLink also joined the training.

## 2.9 VECTOR CONTROL WORKING GROUP MEETING

PMI VectorLink, in collaboration with CNM, organized one Vector Control Working Group meeting on August 26, 2022, in Kampot Province, to bring together all stakeholders who are part of the malaria vector control community in Cambodia to share their implementing plans, best practices, and challenges and to disseminate results. The diversity of the working group members allowed for a rich dialogue and mutual learning to develop more robust and adaptive responses.

# 3. RESULTS

## 3.1 LONGITUDINAL MONITORING

### 3.1.1 VECTOR BIONOMICS

Five bimonthly longitudinal collections were completed from December 2021 to August 2022 across four sentinel annex villages and forest fringes sites in Ou Chay (Stung Treng Province) and Pu Till (Mondulkiri Province) using CDN, HDN, and indoor CDC-LT collection methods.

### 3.1.2 *ANOPHELES* FEMALE MOSQUITOES COLLECTED FROM THE ANNEX VILLAGES AND THE FOREST FRINGES IN THE TWO PROVINCES

*Anopheles* mosquitoes were collected from a total of 30 CDN collections, 60 HDN collections (30 in village sites and 30 in forest fringe sites), and 60 indoor CDC-LT collections in each province (Tables 4 and 5). A total of 12,805 *Anopheles* were collected across both sites and collection methods. CDNs were by far the most productive trapping method accounting for 78.31% (10,027/12,805) of all mosquitoes collected, followed by HDNs at 20.35% (849/12,805) and CDC-LTs light traps at 1.34% (172/12,805). The highest number of different species (species richness) were found in CDNs in both provinces.

**Table 4. Total Number, Density, and Species Richness of *Anopheles* Females in Village and Forest Fringe in Mondulkiri**

| Trapping Method | Number of Traps | Village Site | | | | Number of Traps | Forest Fringe Site | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | *Anopheles* (females) | *Anopheles* (females) per Trap | Number of Species | Number of *Anopheles* Vector Species | | *Anopheles* (females) | *Anopheles* (females) per Trap | Number of Species | Number of *Anopheles* Vector Species |
| CDN | 30 | 6,271 | 209.03 | 24 | *An. dirus* s.l.<br>*An.mimimus* s.l.<br>*An. maculatus* s.l | | NA | | | |
| HDN | 30 | 348 | 11.60 | 17 | *An. dirus* s.l.<br>*An.mimimus* s.l.<br>*An. maculatus* s.l | 30 | 704 | 23.46 | 15 | *An. dirus* s.l.<br>*An.mimimus* s.l.<br>*An. maculatus* s.l |
| CDC-LT | 60 | 86 | 1.43 | 11 | *An. dirus* s.l.<br>*An.mimimus* s.l.<br>*An. maculatus* s.l | | NA | | | |

**Table 5. Total Number, Density, and Species Richness of *Anopheles* Females in Village and Forest Fringe in Stung Treng**

| Trapping Method | Number of Traps | Village Site | | | | Number of Traps | Forest Fringe Site | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | *Anopheles* (females) | *Anopheles* (females) per Trap | Number of Species | Number of *Anopheles* Vector Species | | *Anopheles* (females) | *Anopheles* (females) per Trap | Number of Species | Number of *Anopheles* Vector Species |
| CDN | 27* | 3,756 | 139.11 | 19 | *An. dirus* s.l.<br>*An.mimimus* s.l.<br>*An. maculatus* s.l | | NA | | | |
| HDN | 30 | 501 | 16.70 | 12 | *An. dirus* s.l.<br>*An.mimimus* s.l.<br>*An. maculatus* s.l | 30 | 1,053 | 35.10 | 10 | *An. dirus* s.l.<br>*An.mimimus* s.l.<br>*An. maculatus* s.l |
| CDC-LTs | 60 | 86 | 1.43 | 10 | *An. dirus* s.l.<br>*An. maculatus* s.l | | NA | | | |

*December 2021 collections, only one CDN was deployed.

### 3.1.3 Species Composition and Vector Abundance

#### 3.1.3.1 Total Number and Percentage of Anopheles Females in Annex Village and Forest Fringe Sites in Mondulkiri and Stung Treng Provinces

Thirty-two *Anopheles* species were found in Mondulkiri and Stung Treng provinces (all trapping methods combined) during the five months of collections (Tables 6 and 7).

*An. aconitus* 1,680/6,705 (25.06%) was the most abundant species of the total mosquitoes collected from the annex village site of Mondulkiri. Among the three primary vectors in the province, *An. minimus* s.l. was the most abundant species collected from the village site 850/6705 (12.68%), while *An. dirus* s.l. was most abundant in the forest fringe 359/704 (50.99%). *An. maculatus* s.l. was at 664/7409 (8.96%) in Mondulkiri (Table 6). In Stung Treng, *An. dirus* s.l. was most abundant in both the village 468/4343 (10.78%) and forest fringe 822/1053 (78.10%) (Table 7). Among the twenty-nine other *Anopheles* species captured, three were potential secondary vectors species: *An. barbirostris*, *An. nivipes,* and *An. peditaeniatus*. The other twenty-six species of *Anopheles* mosquitoes collected were *An. philippinensis, An. crawfordi, An. campestris, An. jamesii, An. splendidus, An. vagus, An. indefinitus, An. sawadwongporni* s.l., *An. kochi, An. nigerrimus, An. varuna, An. tessellatus, An. aconitus, An. argyropus, An. annularis, An. baimaii, An. bulkleyi, An. culicifacies, An. karwari, An. letifer, An. nitidus, An. pampanai, An. pseudojamesi, An. subpictus, An. sinensis,* and *An. willmori.*

**Table 6. Total Number, Density, and Species Richness of *Anopheles* Females in Village and Forest Fringe in Mondulkiri (Primary Vectors Highlighted in Gray)**

| Species Name | Village | | Forest | | Total | |
|---|---|---|---|---|---|---|
| | n | % | n | % | N | % |
| *An. aconitus* | 1,680 | 25.06% | 1 | 0.14% | 1,681 | 22.69% |
| *An. minimus* s.l. | 850 | 12.68% | 92 | 13.07% | 942 | 12.71% |
| *An. philippinensis* | 875 | 13.05% | 7 | 0.99% | 882 | 11.90% |
| *An. maculatus* s.l. | 478 | 7.13% | 186 | 26.42% | 664 | 8.96% |
| *An. crawfordi* | 521 | 7.77% | 28 | 3.98% | 549 | 7.41% |
| *An. campestris* | 468 | 6.98% | 1 | 0.14% | 469 | 6.33% |
| *An. barbirostris* | 416 | 6.20% | 5 | 0.71% | 421 | 5.68% |
| *An. dirus* s.l. | 46 | 0.69% | 359 | 50.99% | 405 | 5.47% |
| *An. jamesii* | 240 | 3.58% | 0 | 0.00% | 240 | 3.24% |
| *An. splendidus* | 209 | 3.12% | 6 | 0.85% | 215 | 2.90% |
| *An. vagus* | 179 | 2.67% | 0 | 0.00% | 179 | 2.42% |
| *An. indefinitus* | 169 | 2.52% | 2 | 0.28% | 171 | 2.31% |
| *An. peditaeniatus* | 137 | 2.04% | 1 | 0.14% | 138 | 1.86% |
| *An. sawadwongporni* s.l. | 95 | 1.42% | 5 | 0.71% | 100 | 1.35% |

| Species Name | Village | | Forest | | Total | |
|---|---|---|---|---|---|---|
| | n | % | n | % | N | % |
| *An. nivipes* | 74 | 1.10% | 0 | 0.00% | 74 | 1.00% |
| *An. kochi* | 67 | 1.00% | 1 | 0.14% | 68 | 0.92% |
| *An. nigerrimus* | 19 | 0.28% | 0 | 0.00% | 19 | 0.26% |
| *An. varuna* | 9 | 0.13% | 1 | 0.14% | 10 | 0.13% |
| *An. tessellatus* | 2 | 0.03% | 2 | 0.28% | 4 | 0.05% |
| Other *Anopheles** | 171 | 2.55% | 7 | 0.99% | 178 | 2.40% |
| **Total** | **6,705** | **100%** | **704** | **100%** | **7,409** | **100%** |

*Other *Anopheles* represent a total of 7 different species.

### Table 7. Total Number of *Anopheles* Females in Village and Forest Fringe in Stung Treng (Primary Vectors Highlighted in Gray)

| Species Name | Village | | Forest Fringe | | Total | |
|---|---|---|---|---|---|---|
| | n | % | n | % | N | % |
| *An. dirus* s.l. | 468 | 10.78% | 822 | 78.06% | 1,290 | 23.91% |
| *An. indefinitus* | 1,118 | 25.74% | 0 | 0.00% | 1,118 | 20.72% |
| *An. kochi* | 498 | 11.47% | 106 | 10.07% | 604 | 11.19% |
| *An. vagus* | 533 | 12.27% | 0 | 0.00% | 533 | 9.88% |
| *An. philippinensis* | 488 | 11.24% | 3 | 0.28% | 491 | 9.10% |
| *An. campestris* | 251 | 5.78% | 60 | 5.70% | 311 | 5.76% |
| *An. tessellatus* | 137 | 3.15% | 13 | 1.23% | 150 | 2.78% |
| *An. barbirostris* | 127 | 2.92% | 7 | 0.66% | 134 | 2.48% |
| *An. peditaeniatus* | 108 | 2.49% | 0 | 0.00% | 108 | 2.00% |
| *An. sawadwongporni* s.l. | 98 | 2.26% | 9 | 0.85% | 107 | 1.98% |
| *An. nivipes* | 98 | 2.26% | 1 | 0.09% | 99 | 1.83% |
| *An. maculatus* s.l. | 44 | 1.01% | 7 | 0.66% | 51 | 0.95% |
| *An. nigerrimus* | 27 | 0.62% | 0 | 0.00% | 27 | 0.50% |
| *An. minimus* s.l. | 10 | 0.23% | 3 | 0.28% | 13 | 0.24% |

| Species Name | Village | | Forest Fringe | | Total | |
|---|---|---|---|---|---|---|
| | n | % | n | % | N | % |
| *An. crawfordi* | 6 | 0.14% | 0 | 0.00% | 6 | 0.11% |
| *An. aconitus* | 5 | 0.12% | 0 | 0.00% | 5 | 0.09% |
| *An. jamesii* | 2 | 0.05% | 0 | 0.00% | 2 | 0.04% |
| *An. splendidus* | 1 | 0.02% | 0 | 0.00% | 1 | 0.02% |
| Other *Anopheles** | 324 | 7.46% | 22 | 2.09% | 346 | 6.41% |
| **Total** | **4,343** | **100%** | **1,053** | **100%** | **5,396** | **100%** |

*Other *Anopheles* represent a total of 11 different species.

### 3.1.3.2 Species Composition by Trapping Method in the Mondulkiri Annex Village Site

#### CDNs Set Outdoors (30 traps total, n=6,271)

In comparison to other trapping methods, CDNs in Mondulkiri caught a greater number of *Anopheles* mosquitoes, with a higher species richness (24 *Anopheles* species). *An. aconitus* was the most abundant species (n=1,656, 26.41%), followed by *An. philippinensis* (n=837, 13.35%) (Figure 2A). The primary malaria vectors found in CDNs were as follows: *An. minimus* s.l. (n=794, 12.66%)*, An. maculatus* s.l. (n=447, 7.13%), and *An. dirus* s.l. (n=13, 0.21%). Species that were less than 1% of the total caught are grouped together and represented in Figure 2A as "others." These included *An. nigerrimus, An. dirus* s.l.*, An. varuna, An. tessellatus,* as well as unidentified *Anopheles.* Further details are outlined in Annex 2.

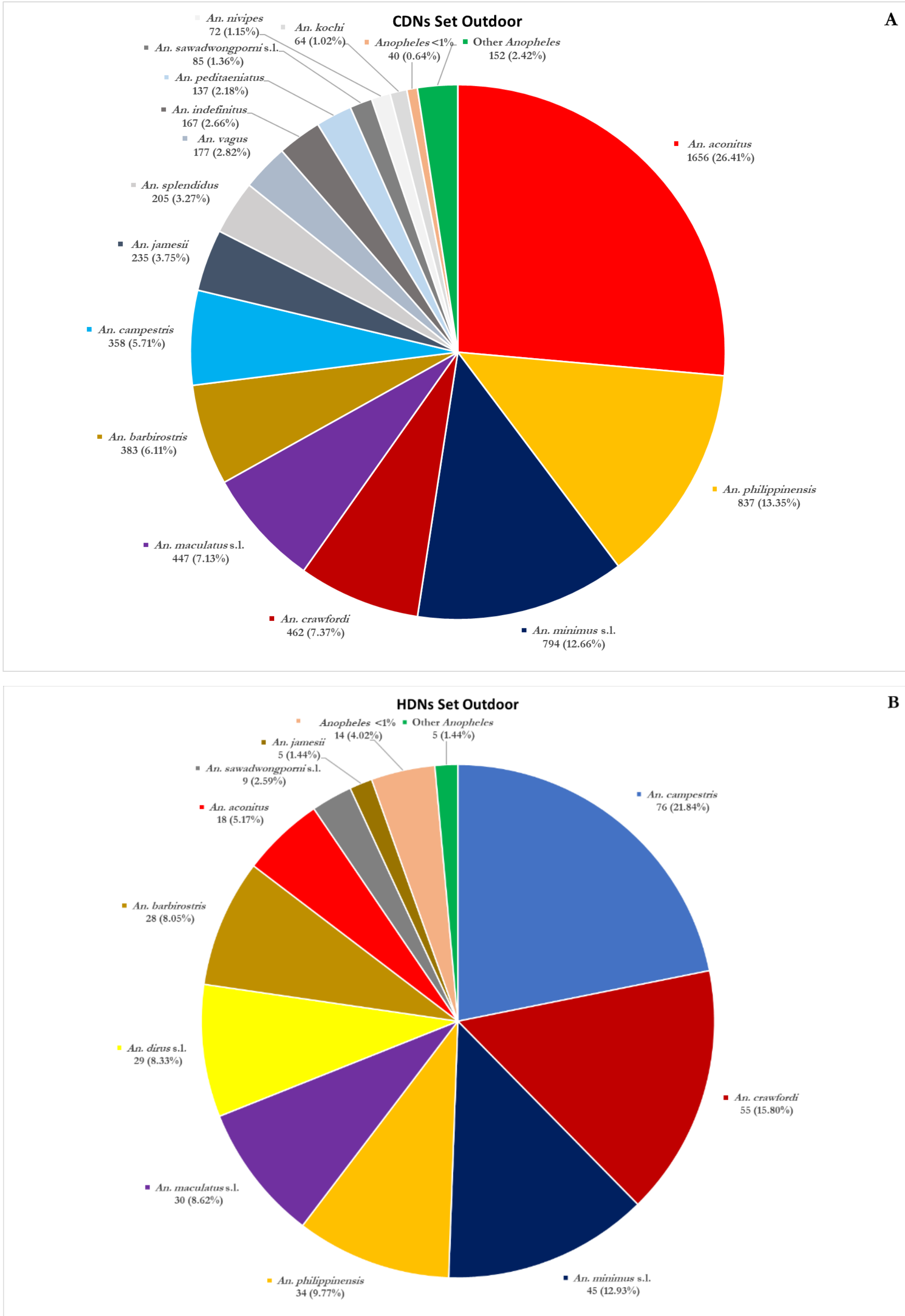
#### HDNs Set Outdoors (30 traps total, n=348)

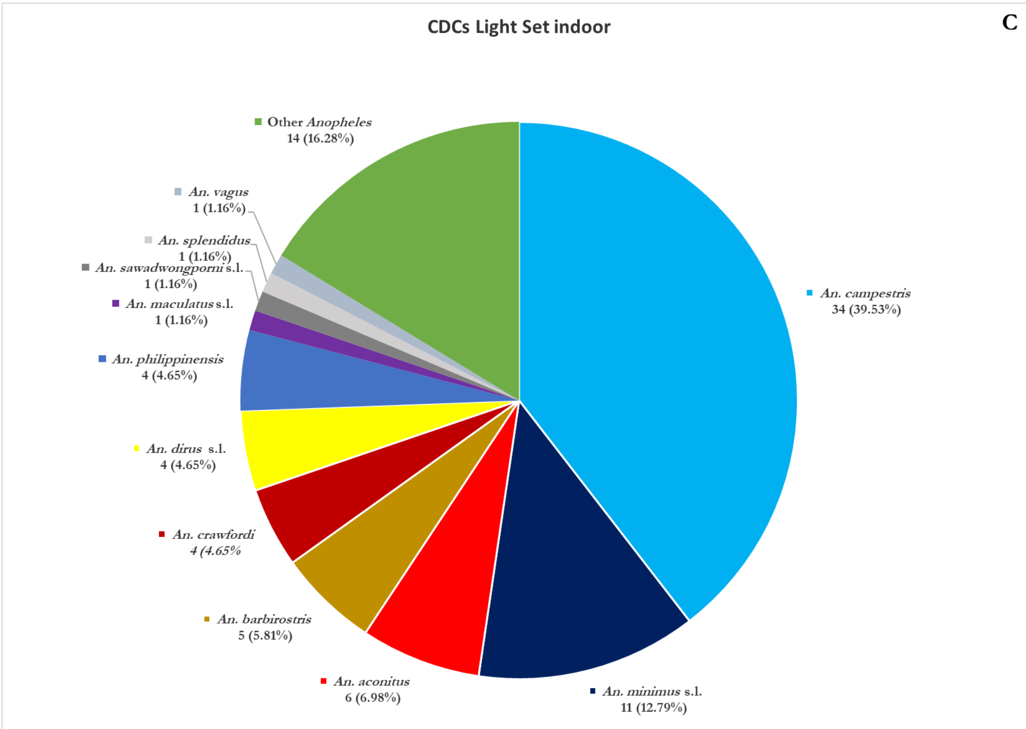
Seventeen *Anopheles* species were caught in HDN collections in the Mondulkiri annex village site (Figure 2B). The most common species was *An. campestris* (n=76, 21.84%) followed by *An. crawfordi* (n=55, 15.80%) and *An. minimus* s.l. (n=45, 12.93%). A total of 30 *An. maculatus* s.l. (8.62%) and 29 *An. dirus* s.l. (8.33%) were collected by HDN in the Mondulkiri annex village. Species that were less than 1% of the total caught are grouped together and represented in Figure 2B as "others." These include *An. kochi, An. splendidus, An. indefinitus, An. nivipes, An. varuna, An. nigerrimus, An. vagus,* as well as unidentified *Anopheles.* Additional information is provided in Annex 3.

#### CDC LT Set Inside Village Houses (60 traps total, n=86)

Eleven *Anopheles* species were collected from the indoor CDC-LT. *An. campestris* was the predominant species found during the collection (n=34, 39.53%). The second most abundant species was one of the primary vectors, *An. minimus* s.l. (n=11, 12.79%), followed by *An. dirus* s.l. (n=4, 4.65%) and *An. maculatus* s.l. (n=1, 1.16%). Species composition is represented in Figure 2C. Further details are listed in Annex 4.

**Figure 2. A-C. *Anopheles* Species Composition from Collections in Pu Til Annex Village, Using A. CDNs Set Outdoors; B. HDNs Set Outdoors; C. CDC Light Traps Set Indoors**



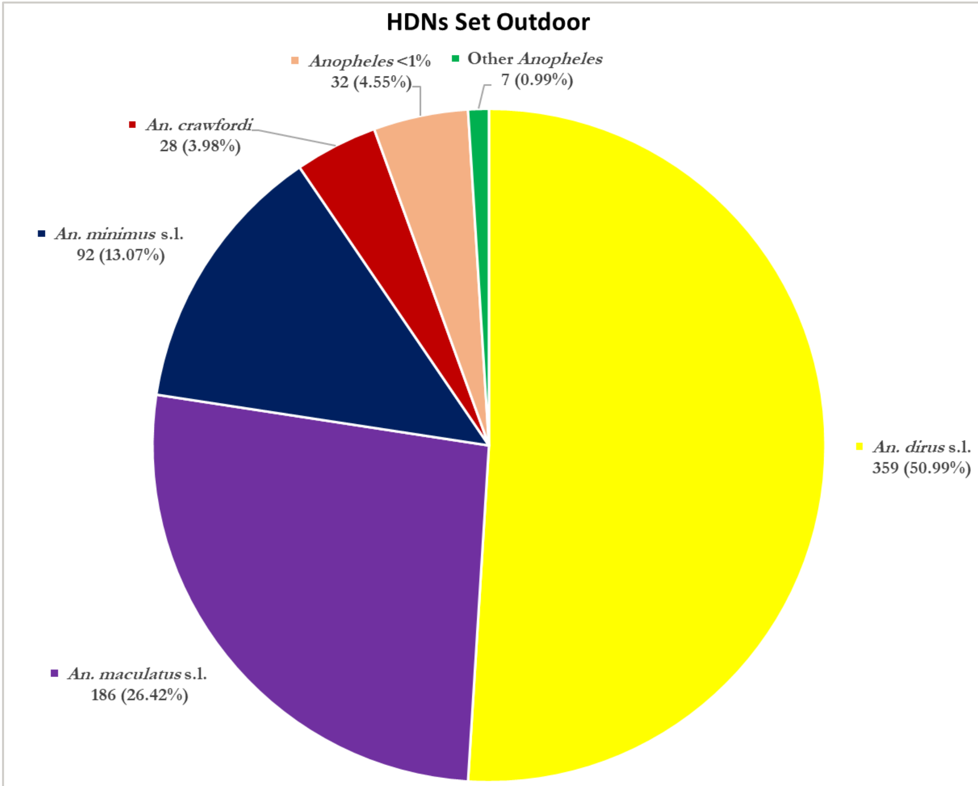



#### 3.1.3.3 Species Composition by Trapping Method in Mondulkiri Forest Fringe Site

#### HDNs Set Outdoors (30 traps total, n=704)

Fifteen *Anopheles* species were caught from the Mondulkiri forest fringe site (Figure 3), using HDNs. The primary malaria vector *An. dirus* s.l. was the most abundant species found in HDNs (n=359, 50.99%) followed by *An. maculatus* s.l. (n=186, 26.42%) and *An. minimus* s.l. (n=92, 13.07%). Species that amounted to less than 1% are grouped together and represented as "others" in Figure 3. They are *An. splendidus, An. barbirostris, An. sawadwongporni* s.l., *An. indefinitus, An. tessellatus, An. aconitus, An. campestris, An. kochi, An. peditaeniatus, An. varuna,* and *An. jamesii.* Additional information is available in Annex 5.

**Figure 3. *Anopheles* Species Composition from HDNs Set Outdoors in the Forest Fringe in Mondulkiri Province**




### 3.1.3.4 Species Composition by Trapping Method in Stung Treng Annex Village Site

#### CDNs Set Outdoors (27 traps total, n=3,756)

Nineteen *Anopheles* species were collected from CDNs in the Stung Treng annex village site (Figure 4A). *An. indefinitus* was the most abundant species in CDNs (n=1,113, 29.63%), followed by *An. vagus* (n=522, 13.90%), *An. philippinensis* (n=478, 12.73%), and *An. kochi* (n=472, 12.57%). The primary malaria vectors found in CDNs were *An. maculatus* s.l. (n=42, 1.12%), *An. dirus* s.l. (n=25, 0.67%), and *An. minimus* s.l. (n=7, 0.19%). Species that made up less than 1% of the total are grouped together as "others" and represented in Figure 4A. These include *An. dirus* s.l., *An. minimus* s.l., *An. nigerrimus, An. crawfordi, An. aconitus, An. jamesii,* and *An. splendidus.* Further details are outlined in Annex 6.

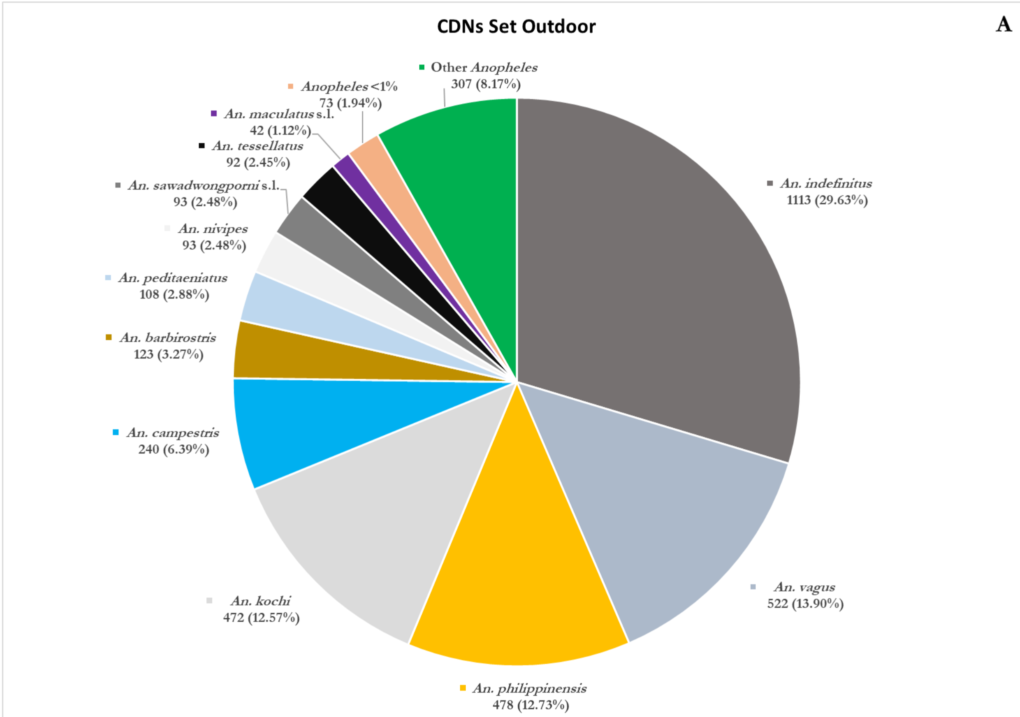
#### HDNs set outdoors (30 traps total, n=501)

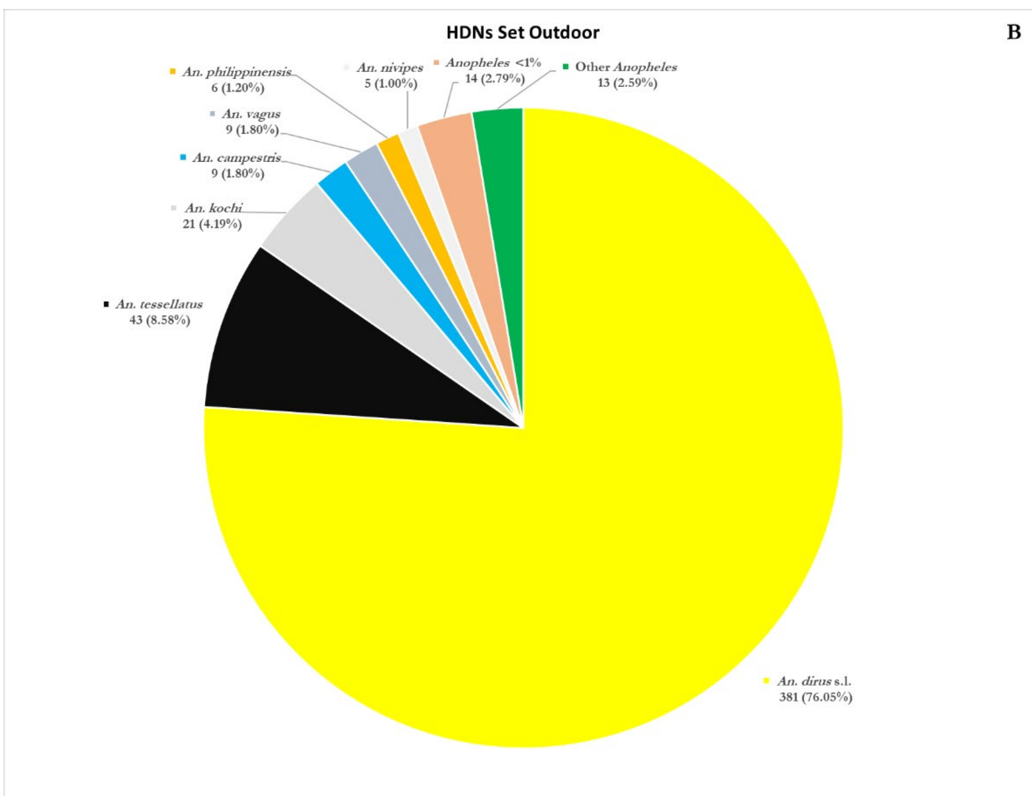
Twelve *Anopheles* species were found in the HDN collections in Stung Treng annex village site (Figure 4B). Primary vector *An. dirus* s.l. was the most abundant species in HDNs (n=381, 76.05%) followed by *An. tessellatus* (n=43, 8.58%). Other primary vectors were caught in very low numbers and included *An. minimus* s.l. (n=3, 0.60%) and *An. maculatus* s.l. (n=1, 0.20%). Species that made up less than 1% of the total are grouped and represented as "others" in Figure 4B. These include *An. maculatus* s.l.*, An. minimus* s.l.*, An. indefinitus, An. barbirostris,* and *An. sawadwongporni* s.l. Additional information is provided in Annex 7.

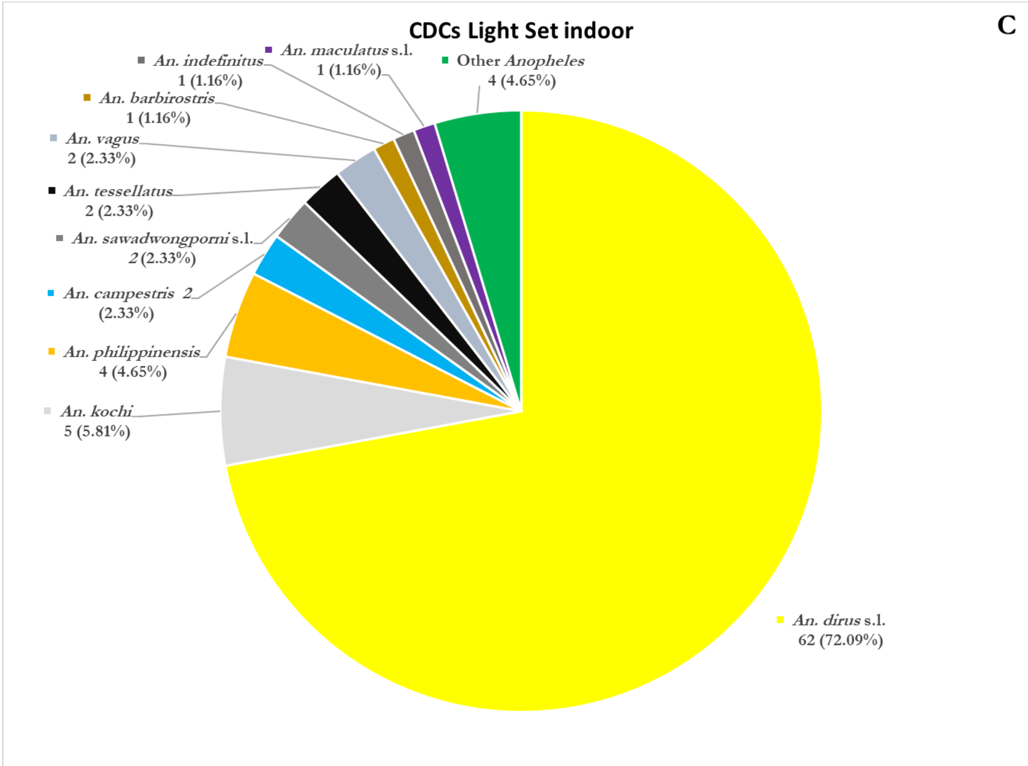
#### CDC Light Trap Set Inside Village Houses (60 traps total, n=86)

Ten *Anopheles* species were collected from the indoor CDC-LT. The primary vector, *An. dirus* s.l. was the predominant species (n=62, 72.09%). *An. maculatus* s.l. was the other primary vector collected. The CDC-LT collected relatively fewer numbers of mosquitoes overall; the species composition is shown in Figure 4C. Additional details are provided in Annex 8.

**Figure 4. A-C. *Anopheles* Species Composition from Collections in Ou Chay Annex Village Using A. CDNs Set Outdoors; B. HDNs; C. CDC Light Traps Set Indoors**



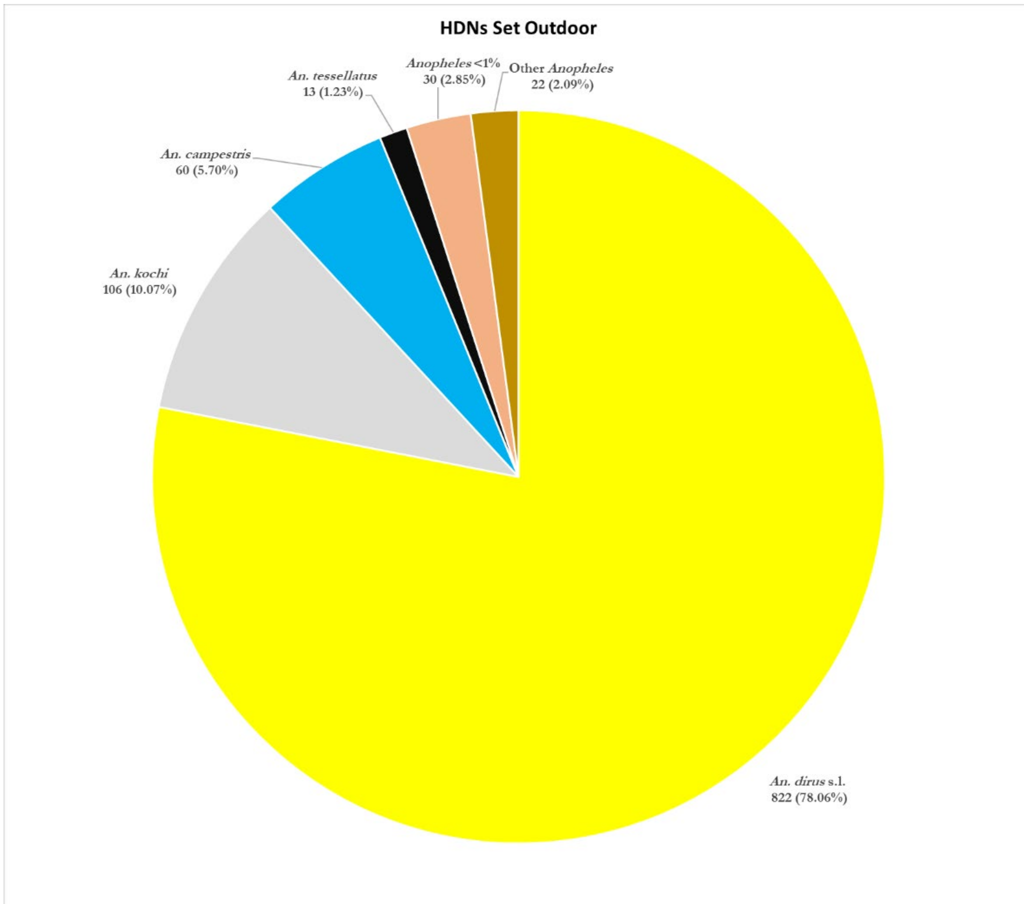



### *3.1.3.5 Species Composition by Trapping Method in Stung Treng Forest Fringe Site*

#### *HDNs in the Forest Fringe (30 traps total, n=1,053)*

Ten *Anopheles* species were caught using HDNs in the Stung Treng forest fringe site (Figure 5). The primary malaria vector *An. dirus* s.l. was the most abundant species found (n=822, 78.06%). The second most abundant species was *An. kochi* (n=106, 10.07%). Other primary malaria vectors, *An. maculatus* s.l. (n=7, 0.66%) and *An. minimus* s.l. (n=3, 0.28%) were also collected in low numbers. Species that made up less than 1% of the total are grouped and represented as “others” in Figure 5. These include *An. sawadwongporni* s.l., *An. barbirosris, An. maculatus* s.l., *An. minimus* s.l., *An. philippinensis,* and *An. nivipus*. The Annex 9 contains further information.

**Figure 5. *Anopheles* Species Composition from HDNs Set Outdoors in the Forest Fringe in Stung Treng Province**




## 3.1.4 Seasonal Biting Rates of the Primary Malaria Vectors

Distinct spatial (between the provinces) and temporal trends in human biting rates of the three primary malaria vectors *An. dirus* s.l.*, An. maculatus* s.l.*,* and *An. minimus* s.l. was apparent.

### *3.1.4.1 Outdoor Human Biting Rates*

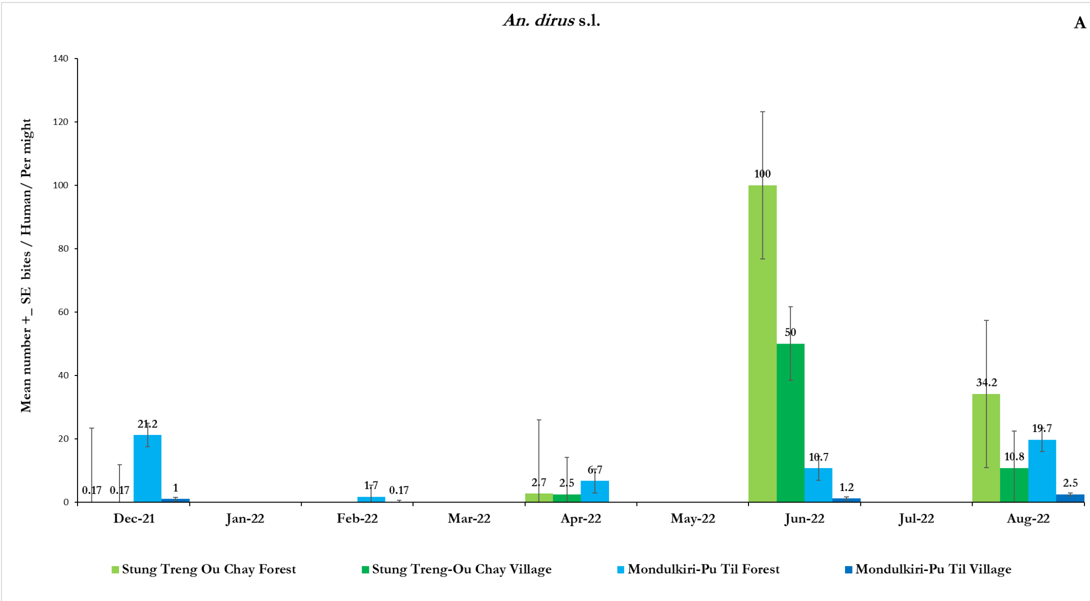
#### *HDNs in Annex Village and Forest Fringe*

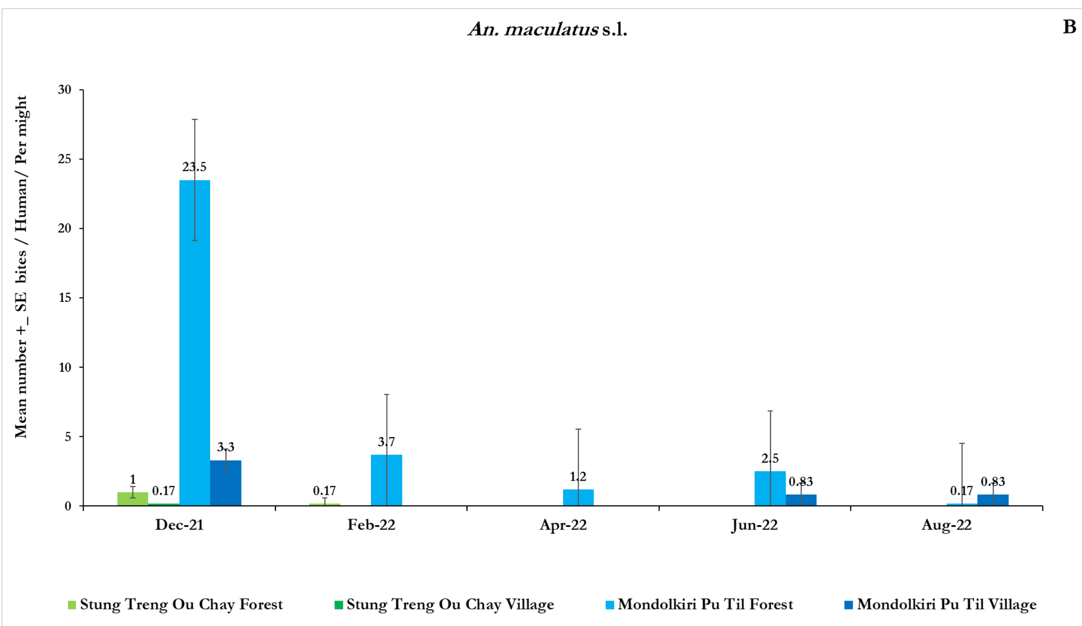
Overall, *An. dirus* s.l. had the highest outdoor human biting rate (HBR), ranging from 0.17 to 100.00 bites per person per night (b/p/n). The peak HBR was recorded in Stung Treng forest (100.00 b/p/n) and village sites (50.00 b/p/n) in June 2022. In Mondulkiri, high HBRs were reported from forest sites in December 2021, with 21.20 b/p/n, and in August 2022, with 19.70 b/p/n (Figure 6A).

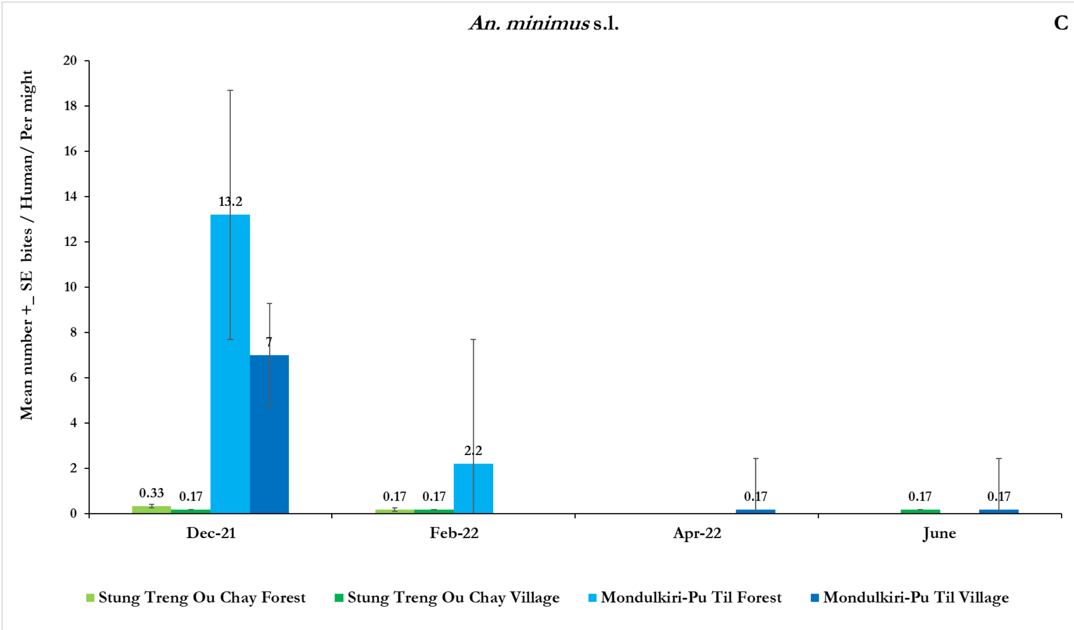
The HBR for *An. maculatus* s.l. differed between the two provinces. Mondulkiri Province had a higher HBR than Stung Treng (Figure 6B). Overall HBR ranged from 0.17 to 23.50 b/p/n. In Mondulkiri, the peak HBRs, 23.50 b/p/n and 3.30 b/p/n, were observed in December 2021 in the village and the forest, respectively. Low HBRs were observed in Stung Treng, ranging from 0.17 to 1.00.

Overall, *An. minimus* s.l. exhibited the lowest HBRs, ranging from 0.17 to 13.20 b/p/n. Similar to *An. maculatus* s.l., the peak HBR (Figure 6C) occurred in December 2021 in the annex village (7.00 b/p/n) and forest fringe sites (13.20 b/p/n) in Mondulkiri Province. The HBR at both the annex village and forest site of the Stung Treng province ranged between 0.17 and 0.33. The peak was observed during December 2021 from the forest site (0.33 b/p/n). Further information can be found in Annex 10&11.

**Figure 6. Mean Human Biting Rate of A. *An. dirus* s.l.; B. *An. maculatus* s.l.; C. *An. minimus* s.l. from HDN in Mondulkiri and Stung Treng Provinces from December 2021 to August 2022**



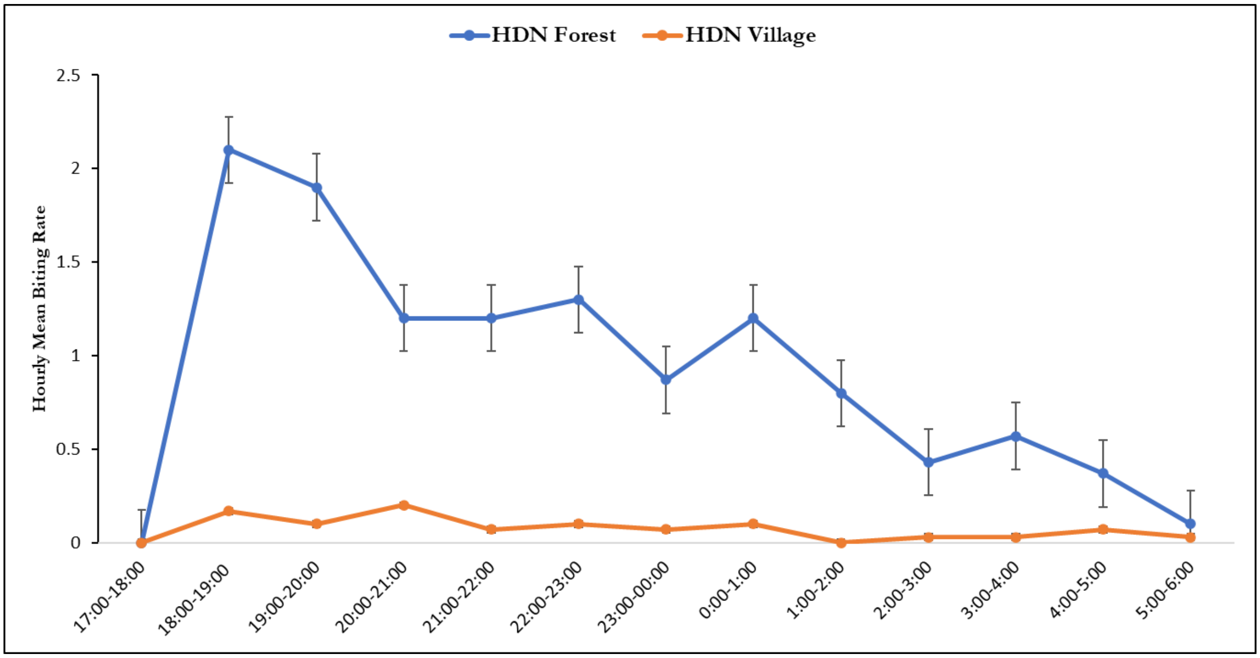


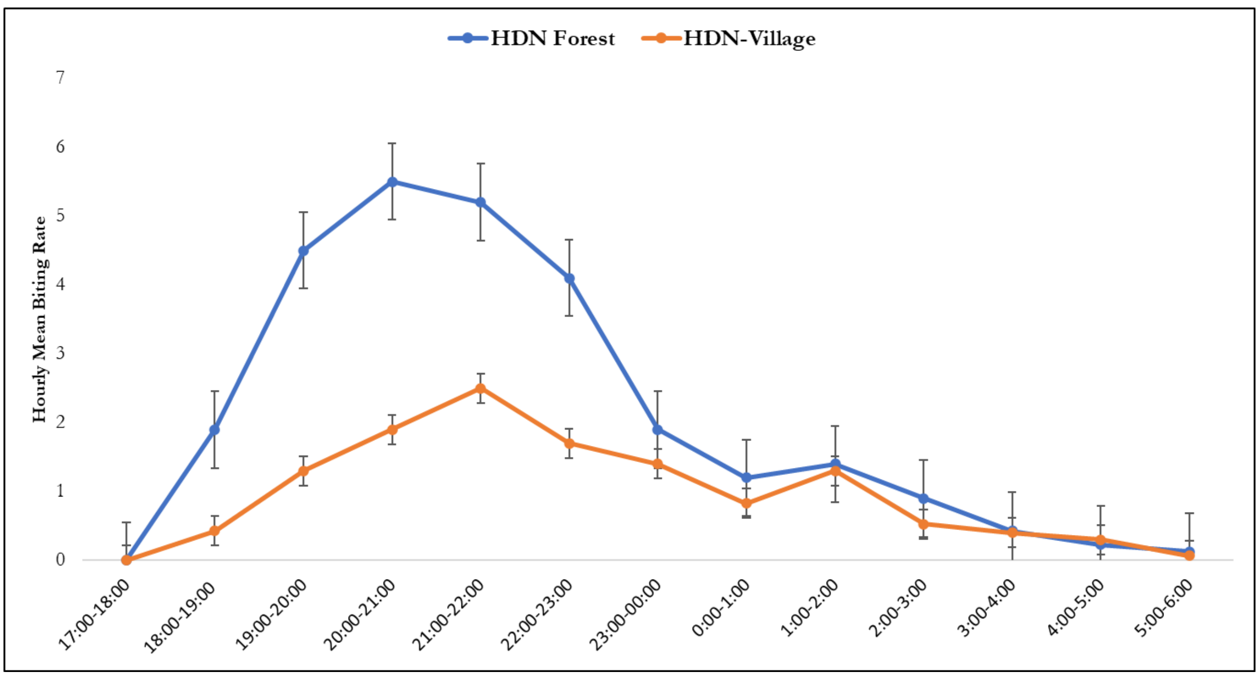

## 3.1.5 Hourly Biting Rates of Primary Malaria Vectors

### 3.1.5.1 *Human Hourly Biting Rates of An. dirus s.l.*

Based on hourly HDN collections, *An. dirus* s.l. was actively biting in Mondulkiri forest from 18:00 and continued biting throughout the night up to 06:00 hours (Figure 7A). This poses potential risk to forest goers who work at night. Peak biting was observed at 18:00 to 19:00, indicating early evening biting. In the village site in Mondulkiri, low numbers of *An. dirus* s.l. were collected and a distinct peak of biting was not evident although low numbers were collected throughout the night. In Stung Treng (Figure 7B) at the forest and village sites, mosquitoes were actively biting from 18:00 and throughout the night up to 06:00 hours. The peak biting time was an hour earlier in the forest (20:00–21:00) than in the village (21:00–22:00). This peak biting time coincides with the sleeping pattern of the people in the same village, where about 11% were found outdoors from 21:00 to 23:00. Further details are outlined in Annexes 12&15.

**Figure 7. Mean Human Biting Rates with Standard Errors of *An. dirus* s.l. in Mondulkiri (top panel) and Stung Treng (bottom panel) Provinces from December 2021 to August 2022**

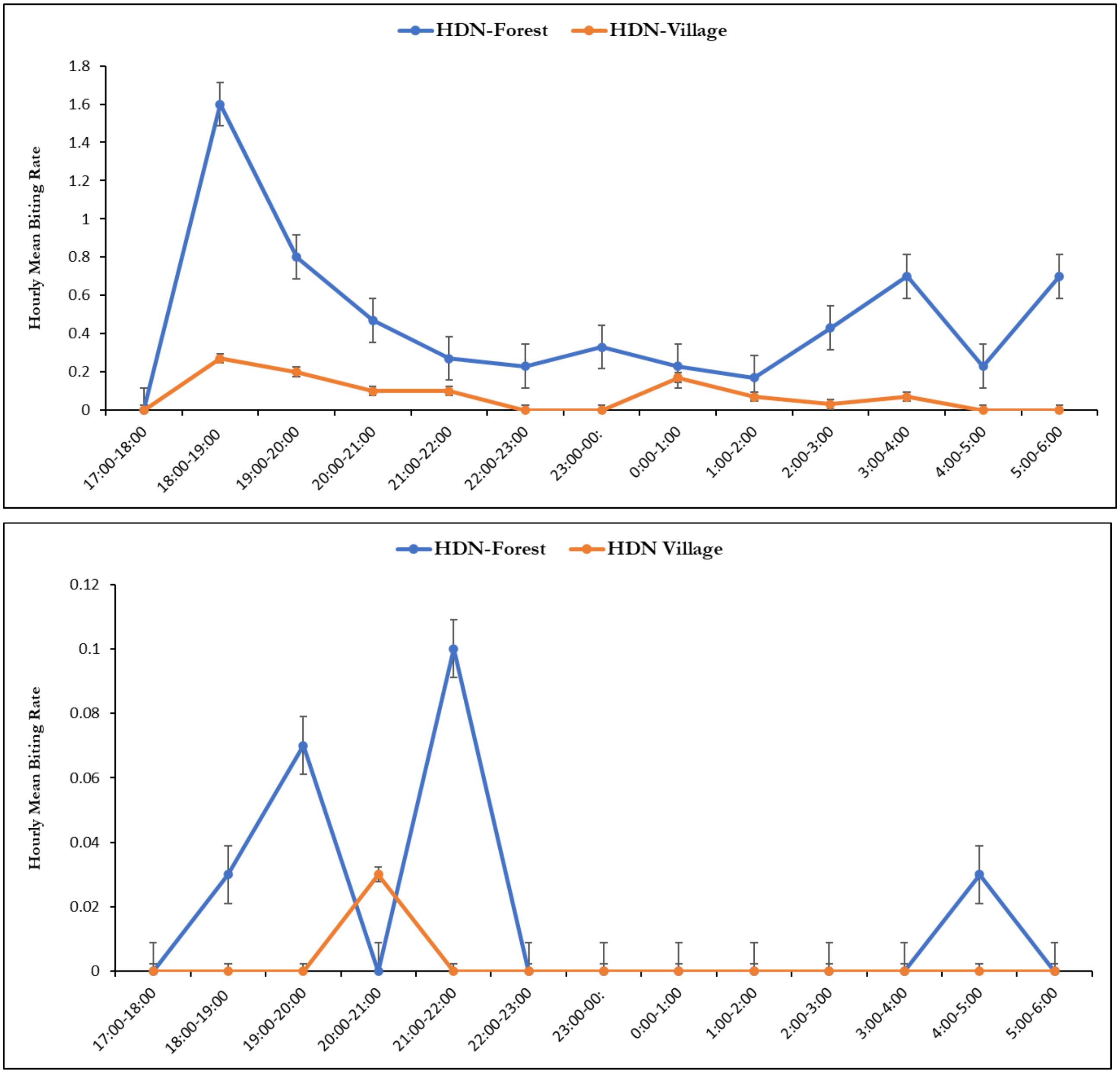



### 3.1.5.2 HUMAN HOURLY BITING RATES OF AN. MACULATUS S.L.

The hourly biting rates of *An. maculatus* s.l. revealed the species bites throughout the night in both Mondulkiri and Stung Treng provinces. The hourly biting rates of *An. maculatus* s.l. were higher in Mondulkiri Province than in Stung Treng Province (Figure 8A). In Stung Treng Province in the forest, due to the low density the biting peak could not be described. (Figure 8B). Additional information is provided in Annex 13 & 16.

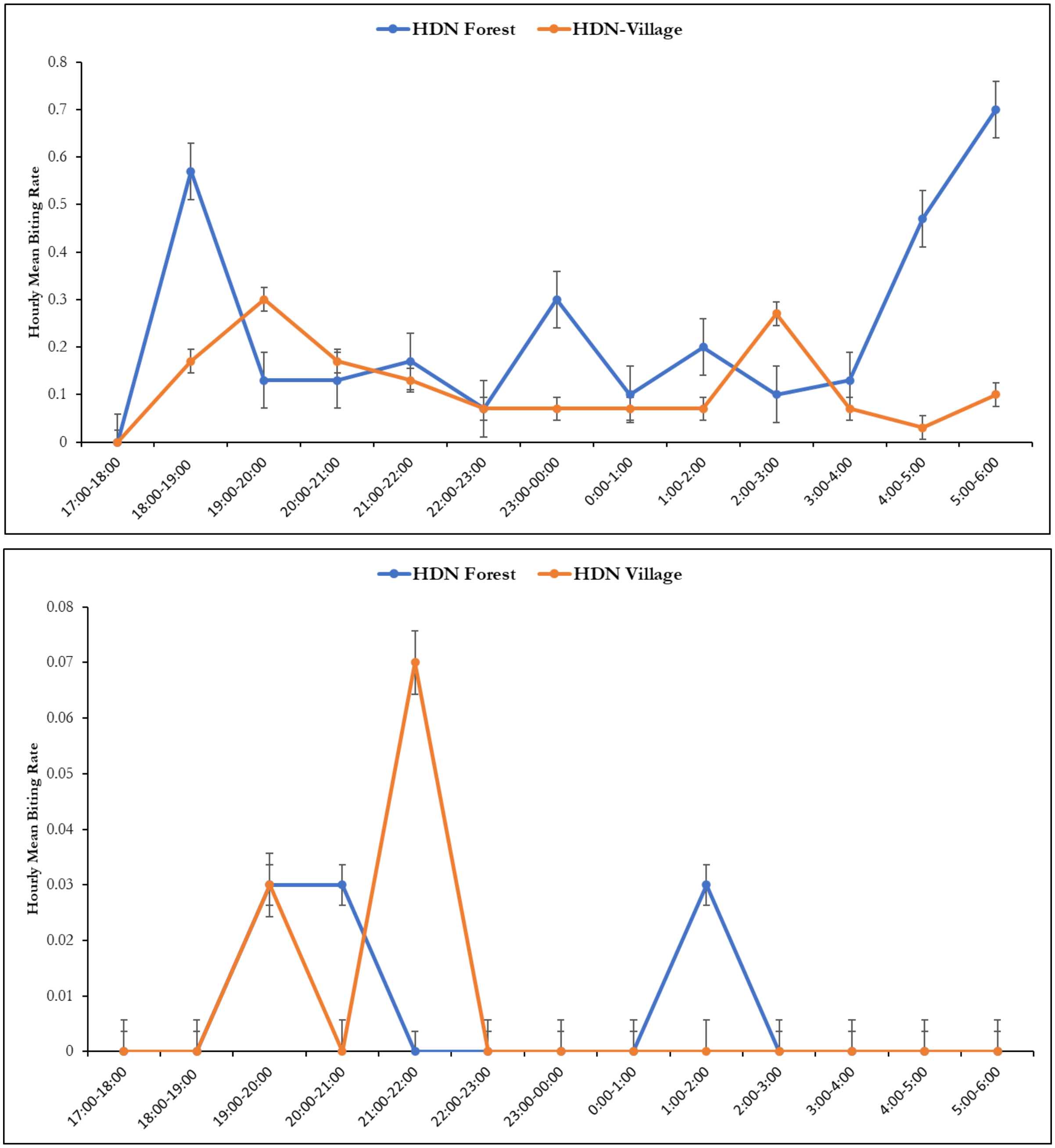
**Figure 8. Mean Human Biting Rates with Standard Errors of *An. maculatus* s.l. in (top panel) Mondulkiri and (bottom panel) Stung Treng Provinces from December 2021 to August 2022**




### 3.1.5.3 HUMAN HOURLY BITING RATES OF *AN. MINIMUS* S.L.

In Mondulkiri, *An. minimus* s.l. were seen actively biting from 18:00 to 06:00 at the forest and village sites. Two peaks were observed in the forest, at 18:00–19:00 and 05:00–06:00 (Figure 9A). In Stung Treng, both village and forest fringe sites biting peak could not be accessed due to low mosquito density (Figure 9B). Further details are listed in Annex 14 & 17.

**Figure 9. Mean Human Biting Rates with Standard Errors of *An. minimus* s.l. in (top panel) Mondulkiri and (bottom panel) Stung Treng Provinces from December 2021 to August 2022**




## 3.1.6 Molecular Analysis of Longitudinal Monitoring Collections

### *3.1.6.1 CS ELISA for Sporozoite Detection*

ELISA was used to test 1,993 *Anopheles vector* mosquitoes which was 59.22% out of all 3365 primary vectors collected from Mondulkiri and Stung Treng provinces from December 2021 to August 2022.

From Mondulkiri, 924 *Anopheles* were tested, and two mosquitoes were found to have *Plasmodium* infection. Among these positives, one was found with the CS antigen of *P. falciparum* and another with *P. vivax*. Out of the 303 *An. dirus* s.l. screened, one was found positive for *P. vivax* (Pv 210). Of the 266 *An. maculatus* s.l.

tested, none were found positive, and out of the 355 *An. minimus* s.l. tested, one was found positive for *P. falciparum*. The infection rates of primary vectors from Mondulkiri were *An. dirus* s.l. 0.33%, *An. minimus* s.l. 0.28% and *An. maculatus* s.l. nil.

From Stung Treng, a total of 1,069 primary vectors were screened *An. dirus* s.l. 1,021, *An. maculatus* s.l. 37, and *An. minimus* s.l. 11. One *An. dirus* s.s. was found with *P. falciparum* infection (infection rate 0.10%) (Table 8).

**Table 8. Number of *Anopheles* Primary Vectors Tested for sporozoites from Mondulkiri and Stung Treng Provinces Collected during Longitudinal Monitoring from December 2021 to August 2022**

| Place of Collection | Morphological Identification | Total Screened | Molecular Identification* | Number Found Positive | Sporozoite Rate |
|---|---|---|---|---|---|
| Mondulkiri | *An. dirus* s.l. | 303 | *An. dirus* s.s. | 1 (*Pv 210*) | 0.33 |
| | *An. maculatus* s.l. | 266 | 0 | 0 | 0.00 |
| | *An. minimus* s.l. | 355 | *An. minimus* s.s. | 1 (*Pf*) | 0.28 |
| Stung Treng | *An. dirus* s.l. | 1,021 | *An. dirus* s.s. | 1 (*Pf*) | 0.10 |
| | *An. maculatus* s.l. | 37 | 0 | 0 | 0.00 |
| | *An. minimus* s.l. | 11 | 0 | 0 | 0.00 |

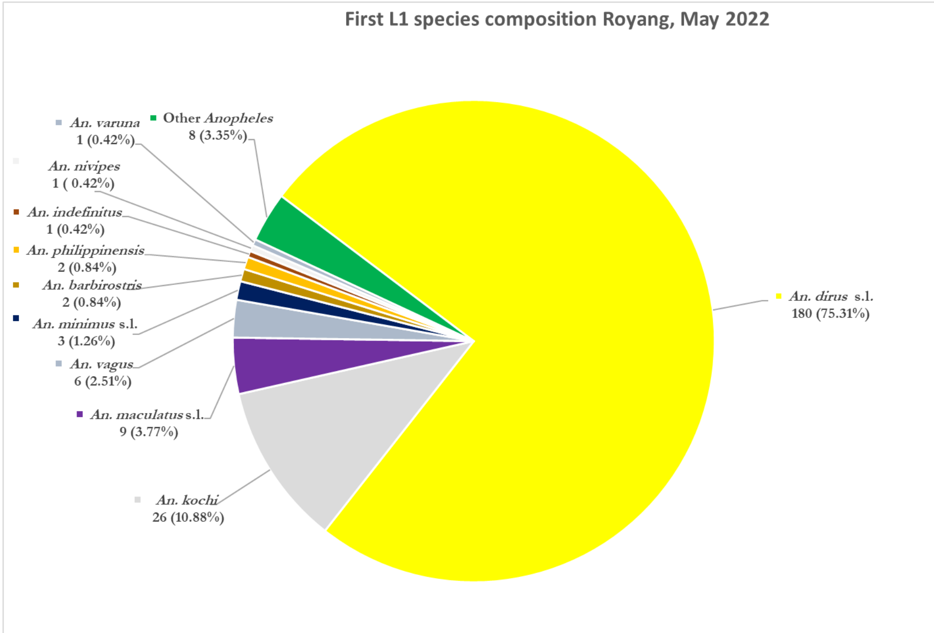
Note: *Pf=Plasmodium falciparum*, *Pv=Plasmodium vivax* (*The molecular analysis for CS ELISA and species confirmation was carried out between July 2022 to March 2023)

## 3.2 CASE-BASED ENTOMOLOGICAL SURVEILLANCE

Six *P. falciparum* L1 foci entomological investigations were conducted from May 2022 to March 2023 in Mondulkiri and Stung Treng provinces. Out of the six foci cases, four were from Mondulkiri and two from Stung Treng.

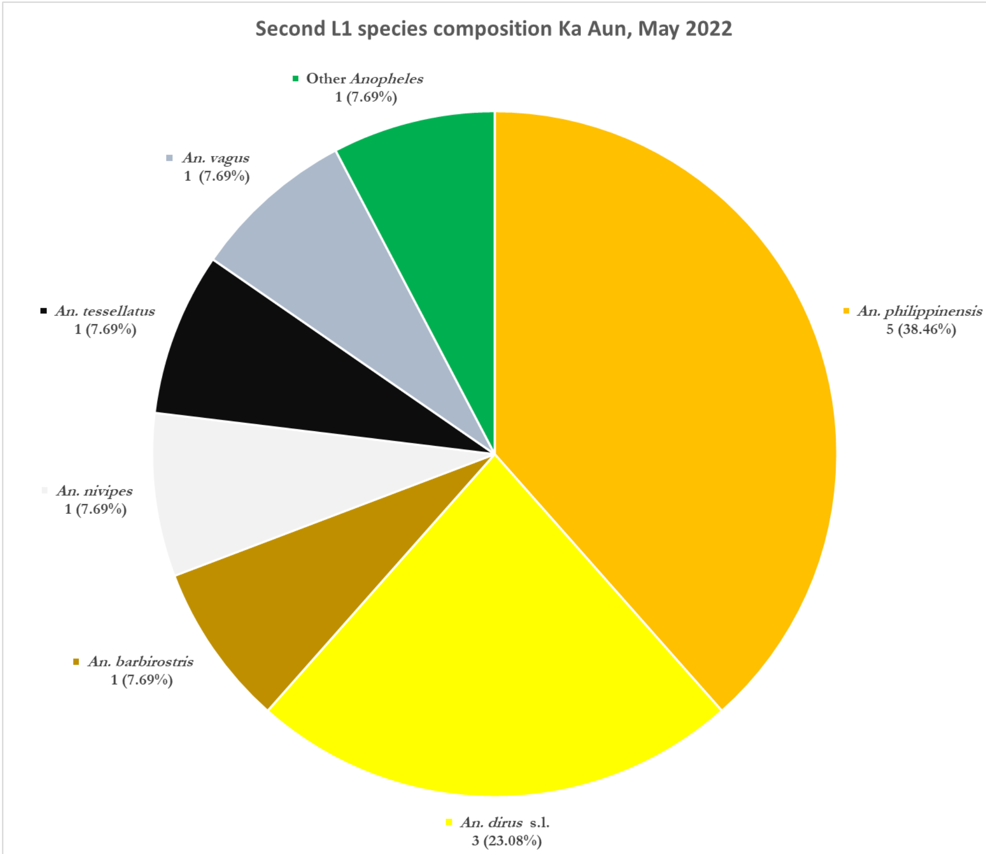
**The first foci** investigation of a 13-year-old boy was conducted in Mondulkiri Province at Royang village (Srae Chhouk Commune, Keo Seima District) in May 2022. All three primary vectors were collected confirming receptivity. *An. dirus* s.l. was the predominant species accounting for 75.31% (180/239) of the total catch, followed by *An. maculatus* s.l. at (9, 3.77%) and *An. mimimus* s.l. at (3, 1.26%). HDNs collected more *An. dirus* s.l. (172/180, 95.55%) than indoor CDC-LTs (8/180, 4.44%). Mosquitoes other than primary vectors collected from both the traps included *An. kochi* 26 (10.90%), *An. vagus* 6 (2.51%), *An. barbirostris* 2 (0.84%), *An. philippinensis* 2 (0.84%), *An. indefinitus* 1 (0.42%), *An. nivipes* 1 (0.42%), *An. varuna* 1 (0.42%), and other unidentified *Anopheles* mosquitoes 8 (3.34%) (Figure 10). Additional information is available in Annex 18. One *An. dirus* s.l. specimen collected in L1 foci investigations tested positive for *P. vivax* 210 and identified molecularly as *An. dirus* s.s. (see 3.2.1 below).

**Figure 10. The First L1; *Anopheles* Species Composition Royang Village, Mondulkiri in May 2022**



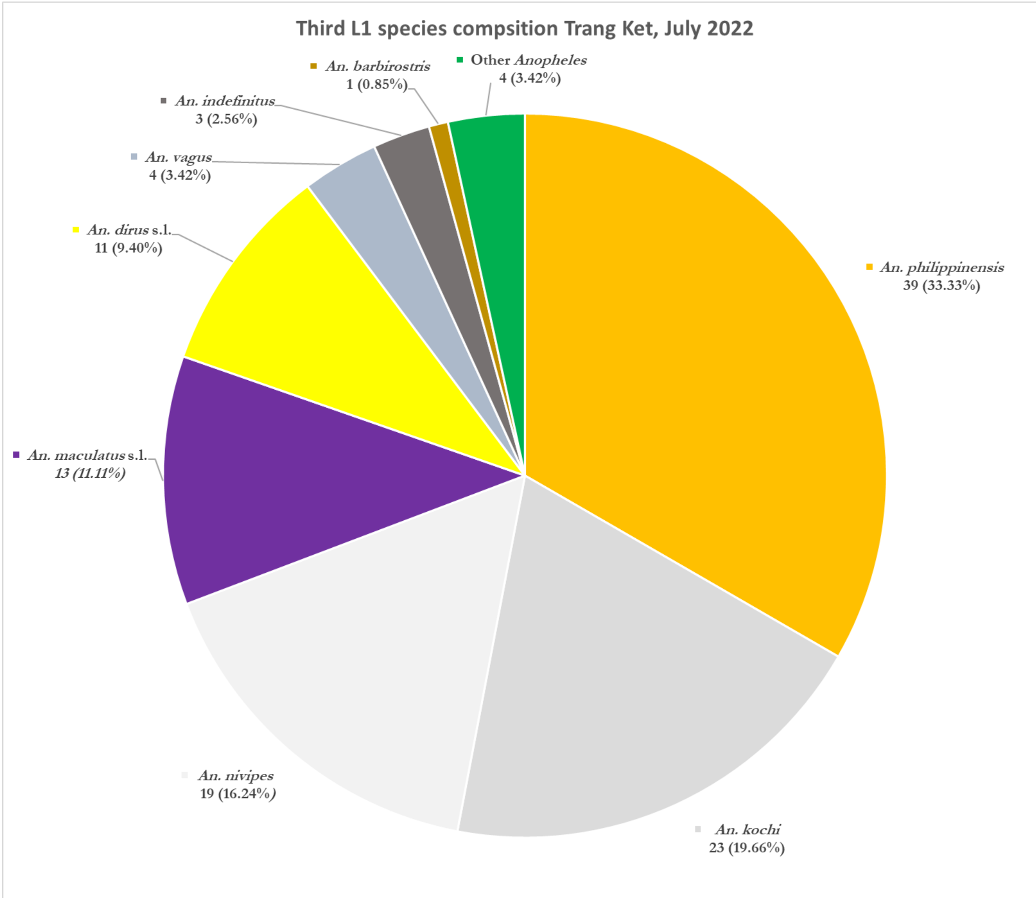

**The second foci** response was also conducted in May 2022. The L1 case was a 10-year-old girl from the Ka Aun annex village of Srae Thom (Sokh Sant Commune, Kaoh Nheaek District of Mondulkiri Province). Receptivity was confirmed by the presence of *An. dirus* s.l. captured indoors by CDC-LT. Surprisingly, the indoor CDC-LT (n=12) collected more *Anopheles* mosquitoes than did the HDN(n=1). The total 13 *Anopheles* mosquitoes caught comprised *An. philippinensis* 5 (38.46%), *An. dirus* s.l. 3 (23.08%), *An. barbirostris* 1 (7.69%), *An. nivipes* 1 (7.69%), *An. tessellatus* 1 (7.69%), *An. vagus* 1 (7.69%), and other unidentified *Anopheles* mosquitoes 1 (7.69%) (Figure 11). Additional details are provided in Annex 19.

**Figure 11. The Second L1; *Anopheles* Species Composition in Ka Aun, Mondulkiri, in May 2022**



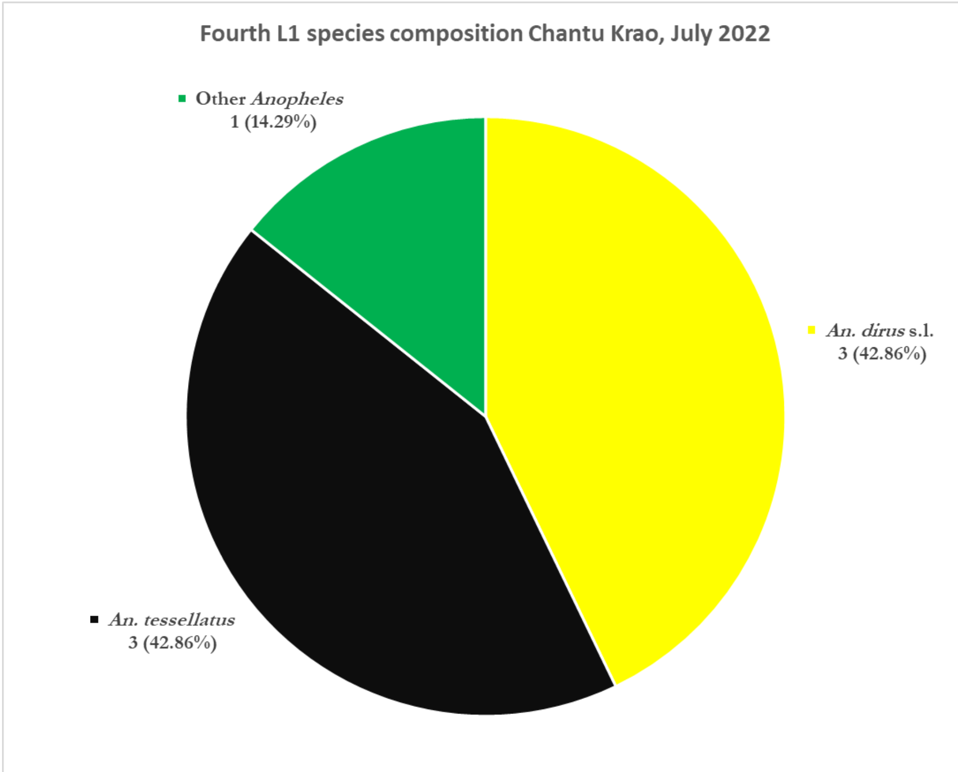

**The third foci** response was conducted in Mondulkiri Province in July 2022. The L1 case was a 38-year-old male from Trang Ket village (Memang Commune, Keo Seima District). During the foci investigation, the primary vectors *An. maculatus* s.l. and *An. dirus* s.l. were captured by both HDN and CDC-LT outdoors and indoors, respectively, confirming receptivity of the focus. Overall, HDNs collected more *Anopheles* mosquitoes than did the indoor CDC-LT. The species composition of the total catch (117 mosquitoes) from the foci village was *An. philippinensis* 39 (33.33%), *An. kochi* 23 (19.66%), *An. nivipes* 19 (16.24%), *An. maculatus* s.l. 13 (11.11%), *An. dirus* s.l. 11 (9.40%), *An. vagus* 4 (3.42%), *An. indefinitus* 3 (2.56%), *An. barbirostris* 1 (0.85%), and other unidentified *Anopheles* mosquitoes 4 (3.42%) (Figure 12). The Annex 20 contains further information.

**Figure 12. The Third L1; *Anopheles* Species Composition in Trang Ket, Mondulkiri, in July 2022**



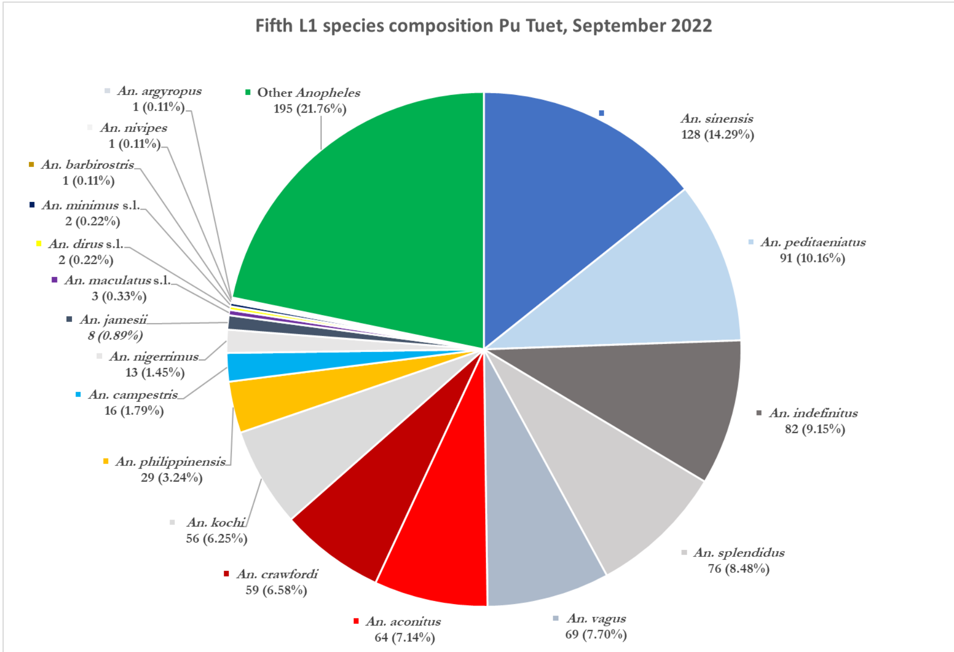

The **fourth foci** response was also in July 2022, but from Stung Treng Province. The L1 case was a 32-year-old female from Chantu Krao Thom village (Sekong Commune, Siem Pang District). *An. dirus* s.l. was the only primary vector captured by both HDN and indoor CDC-LT. The 7 total caught comprised *An. dirus* s.l. 3 (42.86%), *An. tessellatus* 3 (42.86%), and one other unidentified *Anopheles* mosquitoes (14.29%) (Figure 13). Refer to Annex 21 for additional information.

**Figure 13. The Fourth; *Anopheles* Species Composition in Chantu Krao, Stung Treng, in July 2022**



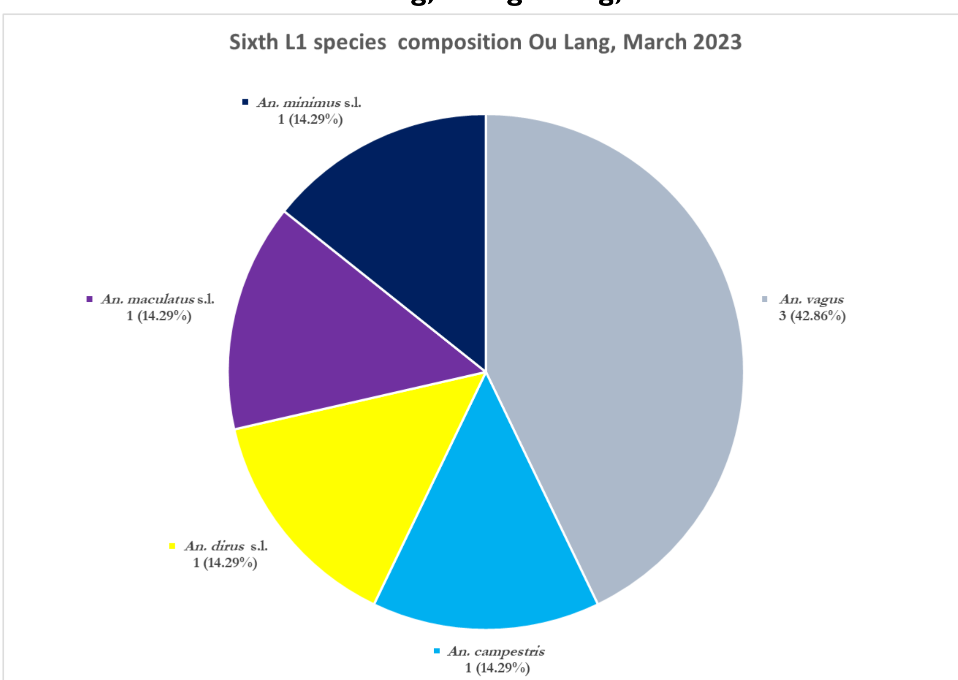

**The fifth foci** response was reported in Mondulkiri Province in September 2022. The L1 case was a 36-year-old male from Pu Tuet village, Bu Sra Commune, Pechreada District. The CDN captured all three primary vectors, *An. minimus* s.l. (n=2) and *An. maculatus* s.l. (n=3), and *An. dirus* s.l. (n=1). HDN caught one *An. dirus* s.l.. Among the 896 other *Anopheles* captured were *An. sinensis* 128 (14.29%), *An. peditaeniatus* 91 (10.16%), *An. indefinitus* 82 (9.15%), *An. splendidus* 76 (8.48%), *An. vagus* 69 (7.70%), *An. aconitus* 64 (7.14%), *An. crawfordi* 59 (6.58%), *An. kochi* 56 (6.25%), *An. philippinensis* 29 (3.24%), *An. campestris* 16 (1.79%), *An. nigerrimus* 13 (1.45%), *An. jamesii* 8 (0.89%), *An. barbirostris* 1 (0.11%), *An. nivipes* 1 (0.11%), *An. argyropus* 1 (0.11%) as unidentified subgenus *Anopheles*, and *Cellia* (Figure 14). Further information can be found in Annex 22.

**Figure 14. The Fifth L1; *Anopheles* Species Composition Captured by all collection methods in Pu Tuet, Mondulkiri, in September 2022**




**The sixth foci** response was reported in Stung Treng Province in March 2023. The L1 case was a seven-year-old female from Ou Lang village, Siem Bouk Commune, Siem Bouk District. Out of three primary vectors, CDN collected *An. minimus* s.l. (n=1) and *An. maculatus* s.l. (n=1), and HDN collected *An. dirus* s.l. (n=1) from outdoors. Among the total of seven *Anopheles* mosquitoes collected, other potential vectors collected included *An. vagus* (n=3) and *An. campestris* (n=1). CDN collected the highest number of *Anopheles* mosquitoes (n=6), followed by HDN (n=1) (Figure 15). Further details are outlined in Annex 23.

**Figure 15. The Sixth L1; *Anopheles* Species Composition Captured by All Collection Methods in Ou Lang, Stung Treng, in March 2023**



The trap wise *Anopheles* primary vectors collection details from Mondulkiri and Stung Treng provinces during six foci investigations are furnished in Annex 24.

### 3.2.1 MOLECULAR ANALYSIS OF FOCI COLLECTIONS

All 231 morphologically identified primary vectors (*An. dirus* s.l. n=199; *An. maculatus* s.l. n=27; *An. minimus* s.l. n=5) collected from six foci investigations from May 2022–March 2023 were processed by CS ELISA for sporozoite detection and PCR for sibling species identification. The CS ELISA results showed that one *An. dirus* s.l., from Royang village in Mondulkiri Province, was found positive for *P. vivax* 210. The PCR screening of this positive mosquito specimen showed it belongs to *An. dirus* s.s. Additional details are provided in Annex 25.

From the six foci investigations, 231 morphologically identified primary vectors were screened through PCR for molecular identification confirmation. Out of the 199 *An. dirus* s.l. analyzed, 82.91% (165) were identified as *An. dirus* s.s. and 17.09% (34) did not amplify. Among the five *An. minimus* s.l. samples screened through PCR, 40.00% (2) were identified as *An. minimus* s.s., 20.00%, (1) as *An. aconitus,* and 40.00% (2) did not amplify. From the 27 morphologically identified *An. maculatus* s.l., 48.00% (13) were identified as *An. sawadwongporni* s.s., 41.00% (11) as *An. rampae* (form K), and 11.00% (3) were unamplified. No *An. maculatus* s.s. could be confirmed. Refer to Annex 26 for additional information.

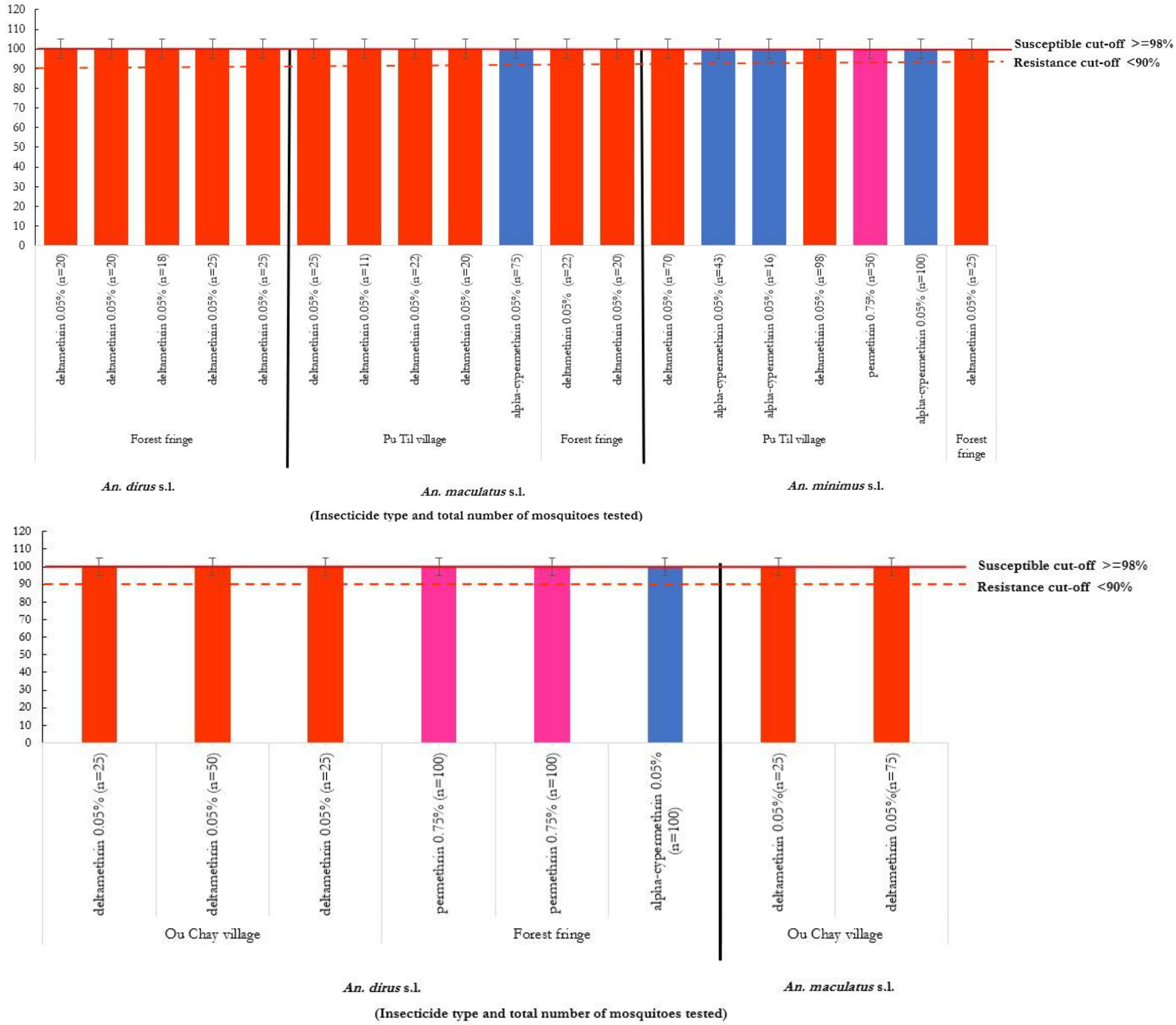
## 3.3 INSECTICIDE RESISTANCE MONITORING

### 3.3.1 INSECTICIDE SUSCEPTIBILITY TESTING FOR PYRETHROID INSECTICIDES

Field-collected adult *An. dirus* s.l. mosquitoes from forest fringe sites of Mondulkiri were susceptible deltamethrin. (Figure 16). *An. maculatus* s.l. from the annex village were susceptible to deltamethrin and alpha-cypermethrin. Additionally, they were also susceptible to deltamethrin from the forest fringe sites. Similarly, *An. minimus* s.l. from the annex village of Mondulkiri Province were susceptible to alpha-cypermethrin, deltamethrin and permethrin, further *An. minimus* s.l. collected from the forest site was susceptible to deltamethrin.

In the Stung Treng province, *An. dirus* s.l. found susceptible to deltamethrin in the annex village and permethrin and alpha-cypermethrin for the forest fringe. *An. maculatus* s.l. from the forest site was found susceptible to deltamethrin. While all the tested vector populations were susceptible, some had sample size less than 100. The sample size ranged from 11 to 100. Due to inadequate mosquito sample size, tests could not be performed for *An. minimus* s.l. from both village and forest sites.

**Figure 16. Insecticide Susceptibility Test to Pyrethroid in the Sites of (top panel) Mondulkiri and (bottom panel) Stung Treng provinces**




## 3.4 HUMAN BEHAVIOR OBSERVATIONS

Human behavioral surveys were conducted in Pu Til annex village in Mondulkiri Province on March 16–17, 2023, and Ou Chay village in Stung Treng on March 25–26, 2023. In Mondulkiri, 31 households were interviewed and information on 103 people was recorded. In Stung Treng, 15 household heads were interviewed and information on 79 people was recorded. Preliminary results of the survey in Mondulkiri indicate that 44% of the respondents were female and 56% were male. Almost all people (95%) slept indoors the previous night before the interview, and only one person did not have an ITN. The remainder (5%) slept outdoors under ITNs. Sixty-five percent of the people went to sleep between 18:00–20:00 hours, 27% between 21:00–23:00, 5% between 00:00–02:00, and 3% between 03:00–05:00.

Preliminary results from Stung Treng indicate that 48% of the respondents were female and 52% were male. Almost all people (97%) slept indoors the previous night while 3% slept outdoors; all of them slept under an ITN. The time to sleep in Pu Til village in Mondulkiri was different from that of Ou Chay in Stung Treng: 89% of people in Ou Chay went to sleep between 18:00–20:00 and 11% between 21:00–23:00. The second phase of the survey will be conducted during the rainy season, when behavior is likely to differ due to the planting season activities.

# 4. OBSERVATIONS AND CONCLUSIONS

In coordination with CNM, VectorLink Cambodia conducted bi-monthly longitudinal entomological surveillance from December 2021 to August 2022 in four sentinel sites, including two village and two forest fringes sites, in Stung Treng and Mondulkiri provinces. The three primary malaria vectors (*An. dirus* s.l.*, An. minimus* s.l., and *An. maculatus* s.l.) were found in varying densities across the sentinel sites monitored. *An. dirus* s.l. was the most abundant species in forest fringes sites in both provinces and in the village sites of Stung Treng Province. In contrast, *An. maculatus* s.l. and *An. minimus* s.l. were found in higher densities in the village site of Mondulkiri Province, compared to the forest fringes sites. This is consistent with the previous year when *An. dirus* s.l. was predominant in the forest sites. The PCR species identification indicates that *An. dirus* s.s., *An. maculatus* s.s., and *An. minimus* s.s. were the most abundant members of these species' complexes. The ELISA sporozoite tests for both longitudinal and foci investigations indicate that *An. minimus* s.s. was found positive for *P. vivax,* while *An. dirus* s.l. was found positive for both *P. falciparum* and *P. vivax*. These results indicate that *P. falciparum* transmission is occurring at the forest sites where it is likely being mediated by *An. dirus* s.l. This poses a challenge for *falciparum* elimination and will require additional vector control interventions targeted toward the forest-going population. It was observed from the current study that in Mondulkiri Province, densities of *An. minimus* s.l. and *An. maculatus* s.l. were much higher in the village than in the forest site during all seasons. The density of *An. maculatus* s.l. and *An. minimus* s.l. was found to be highest in December 2021 in the village and forest sites of Mondulkiri. The *An. dirus* s.l. density was highest in August 2022 and lowest in February 2022. The results show that in Mondulkiri, *An. maculatus* s.l. and *An. minimis* s.l. could play a role in malaria transmission in village areas and a lesser role in forest sites.

Among the three trapping methods used during the reporting period, CDNs caught the highest numbers of *Anopheles* mosquitoes. The CDNs were found to be the most effective trap for capturing *An. maculatus* s.l. and *An. minimus* s.l. from village sites compared to HDNs. Indoor CDC-LTs caught a smaller number of primary vectors indoors as compared to CDNs and HDNs. Nevertheless, they contributed to 12% of primary vectors collected from the village site of Stung Treng, although only 1% from the village site of Mondulkiri. This indicates that though primary vectors are predominantly exophilic, indoor biting does occur and may contribute to persistent indoor malaria transmission. This highlights the need for continued use of ITNs and hammock nets inside houses. Currently, CNM depends exclusively on cow-baited traps for case-based entomological investigations. Since the major objective of these investigations is to determine the presence of primary vectors, CDNs may be limited because they tend to mostly capture zoophilic vectors. Adding HDNs seems beneficial as it enables capture of anthropophilic vectors. These results indicate that all three traps are crucial for entomological surveillance in Cambodia. Hence, it is recommended that whenever resources are available, all three types of traps should be used for entomological monitoring activities including foci investigations.

During the current monitoring period, the HBR of *An. dirus* s.l. was highest in Stung Treng Province in June 2022, during the rainy season, which also coincides with peak malaria transmission season. This also happened during the planting season when most people likely stay at their farmhouses. The biting peak was observed slightly later (December 2021) in Mondulkiri, coinciding with the peak malaria transmission in the province.

The early outdoor biting of all three primary vectors was apparent in both provinces. This undermines the effectiveness of ITNs. The three primary vectors were seen biting actively in the forest in both provinces from 18:00 and throughout the night up to 06:00 hours. Outdoor peak biting time varied between 18:00 and 23:00, coinciding with the sleeping pattern of the people in the villages; approximately 11% of people in

Stung Treng and 35% in Mondulkiri remained outdoors at those times, and hence their being exposed. The all-night biting also poses a potential risk to forest goers who may be working unprotected at night and underlines the need for personal protection tools such as topical repellents.

In view of the current, low-transmission situation in Cambodia, case-based entomological surveillance is essential. Currently, mosquitoes are collected by staff from the OD/health center level and sent to CNM for further identification. However, the process can lead to delays in decision-making, as it is necessary for decision making to conduct foci investigations and disseminate appropriate responses within the shortest timeline possible for an effective outcome. In view of strengthening and sustaining capacity of the country to carry out basic entomological monitoring activities independently as and when required, VectorLink Cambodia, in collaboration with CNM, has trained key local personnel involved in malaria prevention and control on *Anopheles* morphological identification. However, it is important to provide ongoing support and training.

The molecular identification of sibling species indicates relatively high diversity in the *An. maculatus* s.l. group, while fewer species were identified in the *An. dirus* s.l. and *An. minimus* s.l. groups. *An. maculatus* s.l. was also the most difficult species to identify through morphological identification. *An. dirus* s.s. is the primary species found in the *An. dirus* s.l. group and *An. minimus* s.s. in the *An. minimus* s.l. group. In the context of malaria transmission, the identification of sibling species is important. Though morphologically similar, they may differ in biting behavior and susceptibility to insecticides. Incorrect morphological identification can lead to a false receptivity score during foci investigations. Accurate morphological identification shall be ensured through continuous training of OD staff.

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# ANNEX 1. HBS QUESTIONNAIRES

*Checked by: ___________________*
*Checked date: ___________________*

Data collection date: ___________________ Name of data collector: ___________________
Household #___________ Household head initials:___________________ GPS Coordinates:_________________
Province: ___________________ District: ___________________ Commune: ___________________ Village: _________________

| No. | Names (initial or alias) | Residence | | Sex | Age | Sleep, Wake, and Net Use Behaviors | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Please assign numbers to the persons who usually live in your household and guests of the household who stayed here last night, starting with the head of the household. | Does (Person's number) usually live here?<br>CIRCLE YES / NO | Did (Person's number) stay here last night?<br>CIRCLE YES / NO | Is (Person's number) male or female?<br>CIRCLE M / F | How old is (NAME)?<br>RECORD IN YEARS | Did (Person's number) sleep indoors or outdoors last night?<br>CIRCLE IN / OUT | If (Person's number) slept indoors, to the nearest hour, what time last night did (NAME) go indoors?<br>RECORD HOUR ON 24 HOUR CLOCK | To the nearest hour, what time last night did (Person's number) go to sleep?<br>RECORD HOUR ON 24 HOUR CLOCK | Did (Person's number) sleep under a bed net?<br>CIRCLE YES / NO | To the nearest hour, what time last night did (Person's number) wake up and leave bed?<br>RECORD HOUR ON 24 HOUR CLOCK | To the nearest hour, what time last night did (Person's number) go outdoors?<br>RECORD HOUR ON 24 HOUR CLOCK |
| 1 | 2 | 3 | 3.1 | 4 | 5 | 6 | 6.1 | 6.2 | 6.3 | 6.4 | 6.5 |
| 1 | | YES NO | YES NO | M F | | IN OUT | N/A | N/A | YES NO | N/A | N/A |
| 2 | | YES NO | YES NO | M F | | IN OUT | N/A | N/A | YES NO | N/A | N/A |
| 3 | | YES NO | YES NO | M F | | IN OUT | N/A | N/A | YES NO | N/A | N/A |
| 4 | | YES NO | YES NO | M F | | IN OUT | N/A | N/A | YES NO | N/A | N/A |
| 5 | | YES NO | YES NO | M F | | IN OUT | N/A | N/A | YES NO | N/A | N/A |

| No. | Names (initial or alias) | Residence | | Sex | Age | Sleep, Wake, and Net Use Behaviors | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Please assign numbers to the persons who usually live in your household and guests of the household who stayed here last night, starting with the head of the household. | Does (Person's number) usually live here? CIRCLE YES / NO | Did (Person's number) stay here last night? CIRCLE YES / NO | Is (Person's number) male or female? CIRCLE M / F | How old is (NAME)? RECORD IN YEARS | Did (Person's number) sleep indoors or outdoors last night? CIRCLE IN / OUT | If (Person's number) slept indoors, to the nearest hour, what time last night did (NAME) go indoors? RECORD HOUR ON 24 HOUR CLOCK | To the nearest hour, what time last night did (Person's number) go to sleep? RECORD HOUR ON 24 HOUR CLOCK | Did (Person's number) sleep under a bed net? CIRCLE YES / NO | To the nearest hour, what time last night did (Person's number) wake up and leave bed? RECORD HOUR ON 24 HOUR CLOCK | To the nearest hour, what time last night did (Person's number) go outdoors? RECORD HOUR ON 24 HOUR CLOCK |
| 6 | | YES NO | YES NO | M F | | IN OUT | N/A | N/A | YES NO | N/A | N/A |
| 7 | | YES NO | YES NO | M F | | IN OUT | N/A | N/A | YES NO | N/A | N/A |
| 8 | | YES NO | YES NO | M F | | IN OUT | N/A | N/A | YES NO | N/A | N/A |
| 9 | | YES NO | YES NO | M F | | IN OUT | N/A | N/A | YES NO | N/A | N/A |

# ANNEX 2. *ANOPHELES* SPECIES COMPOSITION FROM CDN TRAPS SET OUTDOORS IN THE ANNEX VILLAGE IN MONDULKIRI (30 TRAPS TOTAL) (N=6,271)

| Species Name | CDN-Outdoors Village | |
|---|---|---|
| | Number | Percentage |
| *An. aconitus* | 1,656 | 26.41% |
| *An. philippinensis* | 837 | 13.35% |
| *An. minimus* s.l. | 794 | 12.66% |
| *An. crawfordi* | 462 | 7.37% |
| *An. maculatus* s.l. | 447 | 7.13% |
| *An. barbirostris* | 383 | 6.11% |
| *An. campestris* | 358 | 5.71% |
| *An. jamesii* | 235 | 3.75% |
| *An. splendidus* | 205 | 3.27% |
| *An. vagus* | 177 | 2.82% |
| *An. indefinitus* | 167 | 2.66% |
| *An. peditaeniatus* | 137 | 2.18% |
| *An. sawadwongporni* s.l. | 85 | 1.36% |
| *An. nivipes* | 72 | 1.15% |
| *An. kochi* | 64 | 1.02% |
| *An. nigerrimus* | 18 | 0.29% |

| Species Name | CDN-Outdoors Village | |
|---|---|---|
| | **Number** | **Percentage** |
| *An. dirus* s.l. | 13 | 0.21% |
| *An. varuna* | 7 | 0.11% |
| *An. tessellatus* | 2 | 0.03% |
| Other *Anopheles* | 152 | 2.42% |
| **Total** | **6,271** | **100.00%** |

(Primary vectors highlighter in gray color, Other *Anopheles* represents a total five different species)

# ANNEX 3. *ANOPHELES* SPECIES COMPOSITION FROM HDNS SET OUTDOORS IN THE ANNEX VILLAGE IN MONDULKIRI (30 TRAPS TOTAL) (N=348)

| Species Name | HDN-Outdoors Village | |
|---|---|---|
| | Number | Percentage |
| *An. campestris* | 76 | 21.84% |
| *An. crawfordi* | 55 | 15.80% |
| *An. minimus* s.l. | 45 | 12.93% |
| *An. philippinensis* | 34 | 9.77% |
| *An. maculatus* s.l. | 30 | 8.62% |
| *An. dirus* s.l. | 29 | 8.33% |
| *An. barbirostris* | 28 | 8.05% |
| *An. aconitus* | 18 | 5.17% |
| *An. sawadwongporni* s.l. | 9 | 2.59% |
| *An. jamesii* | 5 | 1.44% |
| *An. kochi* | 3 | 0.86% |
| *An. splendidus* | 3 | 0.86% |
| *An. indefinitus* | 2 | 0.57% |
| *An. nivipes* | 2 | 0.57% |
| *An. varuna* | 2 | 0.57% |
| *An. nigerrimus* | 1 | 0.29% |

| Species Name | HDN-Outdoors Village | |
|---|---|---|
| | **Number** | **Percentage** |
| *An. vagus* | 1 | 0.29% |
| Other *Anopheles* | 5 | 1.44% |
| **Total** | **348** | **100.00%** |

(Primary vectors highlighter in gray color, Other *Anopheles* represents one species)

# ANNEX 4. *ANOPHELES* SPECIES COMPOSITION FROM CDC LIGHT TRAPS SET INDOORS IN THE ANNEX VILLAGE IN MONDULKIRI (60 TRAPS TOTAL) (N=86)

| Species Name | CDC-LTs-Indoors Village | |
|---|---|---|
| | Number | Percentage |
| *An. campestris* | 34 | 39.53% |
| *An. minimus* s.l. | 11 | 12.79% |
| *An. aconitus* | 6 | 6.98% |
| *An. barbirostris* | 5 | 5.81% |
| *An. crawfordi* | 4 | 4.65% |
| *An. dirus* s.l. | 4 | 4.65% |
| *An. philippinensis* | 4 | 4.65% |
| *An. maculatus* s.l. | 1 | 1.16% |
| *An. sawadwongporni* s.l. | 1 | 1.16% |
| *An. splendidus* | 1 | 1.16% |
| *An. vagus* | 1 | 1.16% |
| Other *Anopheles* | 14 | 16.28% |
| **Total** | **86** | **100.00%** |

(Primary vectors highlighter in gray color, Other *Anopheles* represents unidentified up to species level)

# ANNEX 5. *ANOPHELES* SPECIES COMPOSITION FROM HDNS SET OUTDOORS IN THE FOREST FRINGE IN MONDULKIRI (30 TRAPS TOTAL) (N=704)

| Species Name | HDN-Outdoors Forest | |
|---|---|---|
| | Number | Percentage |
| *An. dirus* s.l. | 359 | 50.99% |
| *An. maculatus* s.l. | 186 | 26.42% |
| *An. minimus* s.l. | 92 | 13.07% |
| *An. crawfordi* | 28 | 3.98% |
| *An. philippinensis* | 7 | 0.99% |
| *An. splendidus* | 6 | 0.85% |
| *An. barbirostris* | 5 | 0.71% |
| *An. sawadwongporni* s.l. | 5 | 0.71% |
| *An. indefinitus* | 2 | 0.28% |
| *An. tessellatus* | 2 | 0.28% |
| *An. aconitus* | 1 | 0.14% |
| *An. campestris* | 1 | 0.14% |
| *An. kochi* | 1 | 0.14% |
| *An. peditaeniatus* | 1 | 0.14% |
| *An. varuna* | 1 | 0.14% |

| Species Name | HDN-Outdoors Forest | |
|---|---|---|
| | **Number** | **Percentage** |
| Other *Anopheles* | 7 | 0.99% |
| **Total** | **704** | **100.00%** |

(Primary vectors highlighter in gray color, Other *Anopheles* represents a total two different species)

# ANNEX 6. *ANOPHELES* SPECIES COMPOSITION FROM CDN TRAPS SET OUTDOORS IN THE ANNEX VILLAGE IN STUNG TRENG (27 TRAPS TOTAL) (N=3,756)

| Species Name | CDN-Outdoors Village | |
|---|---|---|
| | Number | Percentage |
| *An. indefinitus* | 1,113 | 29.63% |
| *An. vagus* | 522 | 13.90% |
| *An. philippinensis* | 478 | 12.73% |
| *An. kochi* | 472 | 12.57% |
| *An. campestris* | 240 | 6.39% |
| *An. barbirostris* | 123 | 3.27% |
| *An. peditaeniatus* | 108 | 2.88% |
| *An. nivipes* | 93 | 2.48% |
| *An. sawadwongporni* s.l. | 93 | 2.48% |
| *An. tessellatus* | 92 | 2.45% |
| *An. maculatus* s.l. | 42 | 1.12% |
| *An. dirus sol* | 25 | 0.67% |
| *An. nigerrimus* | 27 | 0.72% |
| *An. crawfordi* | 6 | 0.16% |
| *An. minimus* s.l. | 7 | 0.19% |

| Species Name | CDN-Outdoors Village | |
|---|---|---|
| | Number | Percentage |
| *An. aconitus* | 5 | 0.13% |
| *An. jamesii* | 2 | 0.05% |
| *An. splendidus* | 1 | 0.03% |
| Other *Anopheles* | 307 | 8.17% |
| ***Total*** | **3,756** | **100.00%** |

(Primary vectors highlighter in gray color, Other *Anopheles* represents a total six different species)

# ANNEX 7. *ANOPHELES* SPECIES COMPOSITION FROM HDNS SET OUTDOORS IN THE ANNEX VILLAGE IN STUNG TRENG (30 TRAPS TOTAL) (N=501)

| Species Name | HDN-Outdoors Village | |
|---|---|---|
| | **Number** | **Percentage** |
| *An. dirus* s.l. | 381 | 76.05% |
| *An. tessellatus* | 43 | 8.58% |
| *An. kochi* | 21 | 4.19% |
| *An. campestris* | 9 | 1.80% |
| *An. vagus* | 9 | 1.80% |
| *An. philippinensis* | 6 | 1.20% |
| *An. nivipes* | 5 | 1.00% |
| *An. indefinitus* | 4 | 0.80% |
| *An. barbirostris* | 3 | 0.60% |
| *An. minimus* s.l. | 3 | 0.60% |
| *An. sawadwongporni* s.l. | 3 | 0.60% |
| *An. maculatus* s.l. | 1 | 0.20% |
| Other *Anopheles* | 13 | 2.59% |
| **Total** | **501** | **100.00%** |

(Primary vectors highlighter in gray, Other *Anopheles* represents a total three different species)

# Annex 8. *Anopheles* Species Composition from CDC Light Traps Set Indoors in the Annex Village in Stung Treng (60 traps total) (n=86)

| Species Name | CDC-LTs-Indoors Village | |
|---|---|---|
| | Number | Percentage |
| *An. dirus* s.l. | 62 | 72.09% |
| *An. kochi* | 5 | 5.81% |
| *An. philippinensis* | 4 | 4.65% |
| *An. campestris* | 2 | 2.33% |
| *An. sawadwongporni* s.l. | 2 | 2.33% |
| *An. tessellatus* | 2 | 2.33% |
| *An. vagus* | 2 | 2.33% |
| *An. barbirostris* | 1 | 1.16% |
| *An. indefinitus* | 1 | 1.16% |
| *An. maculatus* s.l. | 1 | 1.16% |
| Other *Anopheles* | 4 | 4.65% |
| **Total** | **86** | **100.00%** |

(Primary vectors highlighter in gray, Other *Anopheles* represents unidentified up to species level)

# ANNEX 9. *ANOPHELES* SPECIES COMPOSITION FROM HDNS SET OUTDOORS IN THE FOREST FRINGE IN STUNG TRENG (30 TRAPS TOTAL) (N=1,053)

| Species Name | HDN-Outdoors Forest | |
|---|---|---|
| | Number | Percentage |
| *An. dirus* s.l. | 822 | 78.06% |
| *An. kochi* | 106 | 10.07% |
| *An. campestris* | 60 | 5.70% |
| *An. tessellatus* | 13 | 1.23% |
| *An. sawadwongporni* s.l. | 9 | 0.85% |
| *An. barbirostris* | 7 | 0.66% |
| *An. maculatus* s.l. | 7 | 0.66% |
| *An. minimus* s.l. | 3 | 0.28% |
| *An. philippinensis* | 3 | 0.28% |
| *An. nivipes* | 1 | 0.09% |
| Other *Anopheles* | 22 | 2.09% |
| **Total** | **1,053** | **100.00%** |

(Primary vectors highlighter in gray, Other *Anopheles* represents a total five different species)

# Annex 10. Mean Human Biting Rates of Primary Malaria Vectors (*An. dirus* s.l., *An. maculatus* s.l., *and An. minimus* s.l.) from HDNs in Sentinel Villages of Mondulkiri and Stung Treng Provinces from December 2021 to August 2022

| Month | *An. dirus* s.l. | | *An. maculatus* s.l. | | *An. minimus* s.l. | |
|---|---|---|---|---|---|---|
| | **Mondulkiri** | **Stung Treng** | **Mondulkiri** | **Stung Treng** | **Mondulkiri** | **Stung Treng** |
| December 2021 | 1.00 | 0.17 | 3.30 | 0.17 | 7.00 | 0.17 |
| February 2022 | 0.17 | 0.00 | 0.00 | 0.00 | 0.00 | 0.17 |
| April 2022 | 0.00 | 2.50 | 0.00 | 0.00 | 0.17 | 0.00 |
| June 2022 | 1.20 | 50.00 | 0.83 | 0.00 | 0.17 | 0.17 |
| August 2022 | 2.50 | 10.80 | 0.83 | 0.00 | 0.17 | 0.00 |

# Annex 11. Mean Human Biting Rates of Primary Malaria Vectors (*An. dirus* s.l., *An. maculatus* s.l., *and An. minimus* s.l.) from HDN in forest sites of Mondulkiri and Stung Treng Provinces from December 2021 to August 2022

| Month | *An. dirus* s.l. | | *An. maculatus* s.l. | | *An. minimus* s.l. | |
|---|---|---|---|---|---|---|
| | **Mondulkiri** | **Stung Treng** | **Mondulkiri** | **Stung Treng** | **Mondulkiri** | **Stung Treng** |
| December 2021 | 21.20 | 0.17 | 23.50 | 1.00 | 13.20 | 0.33 |
| February 2022 | 1.70 | 0.00 | 3.70 | 0.17 | 2.20 | 0.17 |
| April 2022 | 6.70 | 2.70 | 1.20 | 0.00 | 0.00 | 0.00 |
| June 2022 | 10.70 | 100.00 | 2.50 | 0.00 | 0.00 | 0.00 |
| August 2022 | 19.70 | 34.20 | 0.17 | 0.00 | 0.00 | 0.00 |

# Annex 12. Mean Hourly Biting Rate of *An. dirus* s.l. from HDN, in Village and Forest Fringe Sites in Mondulkiri Province from December 2021 to August 2022

| Hour | HDN | | | |
|---|---|---|---|---|
| | Number Caught (annex village) (36 nights) | Biting Rate (Village) | Number Caught (forest fringe) (36 nights) | Biting Rate (forest fringe) |
| 17:00–18:00 | 0 | 0.00 | 0 | 0.00 |
| 18:00–19:00 | 5 | 0.17 | 64 | 2.10 |
| 19:00–20:00 | 3 | 0.10 | 56 | 1.90 |
| 20:00–21:00 | 6 | 0.20 | 35 | 1.20 |
| 21:00–22:00 | 2 | 0.07 | 36 | 1.20 |
| 22:00–23:00 | 3 | 0.10 | 38 | 1.30 |
| 23:00–00:00 | 2 | 0.07 | 26 | 0.87 |
| 00:00–01:00 | 3 | 0.10 | 36 | 1.20 |
| 01:00–02:00 | 0 | 0.00 | 24 | 0.80 |
| 02:00–03:00 | 1 | 0.03 | 13 | 0.43 |
| 03:00–04:00 | 1 | 0.03 | 17 | 0.57 |
| 04:00–05:00 | 2 | 0.07 | 11 | 0.37 |
| 05:00–06:00 | 1 | 0.03 | 3 | 0.10 |

# Annex 13. Mean Hourly Biting Rate of *An. maculatus* s.l. from HDN in Village and Forest Fringe Sites in Mondulkiri Province from December 2021 to August 2022

| Hour | HDN | | | |
|---|---|---|---|---|
| | Number Caught (annex village) (36 nights) | Biting Rate (Village) | Number Caught (forest fringe) (36 nights) | Biting Rate (forest fringe) |
| 17:00–18:00 | 0 | 0.00 | 0 | 0.00 |
| 18:00–19:00 | 8 | 0.27 | 49 | 1.60 |
| 19:00–20:00 | 6 | 0.20 | 24 | 0.80 |
| 20:00–21:00 | 3 | 0.10 | 14 | 0.47 |
| 21:00–22:00 | 3 | 0.10 | 8 | 0.27 |
| 22:00–23:00 | 0 | 0.00 | 7 | 0.23 |
| 23:00–00:00 | 0 | 0.00 | 10 | 0.33 |
| 00:00–01:00 | 5 | 0.17 | 7 | 0.23 |
| 01:00–02:00 | 2 | 0.07 | 5 | 0.17 |
| 02:00–03:00 | 1 | 0.03 | 13 | 0.43 |
| 03:00–04:00 | 2 | 0.07 | 21 | 0.70 |
| 04:00–05:00 | 0 | 0.00 | 7 | 0.23 |
| 05:00–06:00 | 0 | 0.00 | 21 | 0.70 |

# Annex 14. Mean Hourly Biting Rate of *An. minimus* s.l. from HDN in Village and Forest Fringe Sites in Mondulkiri Province from December 2021 to August 2022

| Hour | HDN | | | |
|---|---|---|---|---|
| | Number Caught (annex village) (36 nights) | Biting Rate (Village) | Number Caught (forest fringe) (36 nights) | Biting Rate (forest fringe) |
| 17:00–18:00 | 0 | 0.00 | 0 | 0.00 |
| 18:00–19:00 | 5 | 0.17 | 17 | 0.57 |
| 19:00–20:00 | 9 | 0.30 | 4 | 0.13 |
| 20:00–21:00 | 5 | 0.17 | 4 | 0.13 |
| 21:00–22:00 | 4 | 0.13 | 5 | 0.17 |
| 22:00–23:00 | 2 | 0.07 | 2 | 0.07 |
| 23:00–00:00 | 2 | 0.07 | 9 | 0.30 |
| 00:00–01:00 | 2 | 0.07 | 3 | 0.10 |
| 01:00–02:00 | 2 | 0.07 | 6 | 0.20 |
| 02:00–03:00 | 8 | 0.27 | 3 | 0.10 |
| 03:00–04:00 | 2 | 0.07 | 4 | 0.13 |
| 04:00–05:00 | 1 | 0.03 | 14 | 0.47 |
| 05:00–06:00 | 3 | 0.10 | 21 | 0.70 |

# Annex 15. Mean Hourly Biting Rate of *An. dirus* s.l. from HDN in Village and Forest Fringe Sites in Stung Treng Province from December 2021 to August 2022

| Hour | HDN | | | |
|---|---|---|---|---|
| | **Number Caught (annex village) (36 nights)** | **Biting Rate (Village)** | **Number Caught (forest fringe) (36 nights)** | **Biting Rate (forest fringe)** |
| 17:00–18:00 | 0 | 0.00 | 0 | 0.00 |
| 18:00–19:00 | 13 | 0.43 | 57 | 1.90 |
| 19:00–20:00 | 38 | 1.30 | 136 | 4.50 |
| 20:00–21:00 | 57 | 1.90 | 164 | 5.50 |
| 21:00–22:00 | 76 | 2.50 | 157 | 5.20 |
| 22:00–23:00 | 51 | 1.70 | 122 | 4.10 |
| 23:00–00:00 | 43 | 1.40 | 56 | 1.90 |
| 00:00–01:00 | 25 | 0.83 | 37 | 1.20 |
| 01:00–02:00 | 39 | 1.30 | 42 | 1.40 |
| 02:00–03:00 | 16 | 0.53 | 27 | 0.90 |
| 03:00–04:00 | 12 | 0.40 | 13 | 0.43 |
| 04:00–05:00 | 9 | 0.30 | 7 | 0.23 |
| 05:00–06:00 | 2 | 0.07 | 4 | 0.13 |

# Annex 16. Mean Hourly Biting Rate of *An. maculatus* s.l. from HDN in Village and Forest Fringe Sites in Stung Treng Province from December 2021 to August 2022

| Hour | HDN | | | |
|---|---|---|---|---|
| | Number Caught (annex village) (36 nights) | Biting Rate (Village) | Number Caught (forest fringe) (36 nights) | Biting Rate (forest fringe) |
| 17:00–18:00 | 0 | 0.00 | 0 | 0.00 |
| 18:00–19:00 | 0 | 0.00 | 1 | 0.03 |
| 19:00–20:00 | 0 | 0.00 | 2 | 0.07 |
| 20:00–21:00 | 1 | 0.03 | 0 | 0.00 |
| 21:00–22:00 | 0 | 0.00 | 3 | 0.10 |
| 22:00–23:00 | 0 | 0.00 | 0 | 0.00 |
| 23:00–00:00 | 0 | 0.00 | 0 | 0.00 |
| 00:00–01:00 | 0 | 0.00 | 0 | 0.00 |
| 01:00–02:00 | 0 | 0.00 | 0 | 0.00 |
| 02:00–03:00 | 0 | 0.00 | 0 | 0.00 |
| 03:00–04:00 | 0 | 0.00 | 0 | 0.00 |
| 04:00–05:00 | 0 | 0.00 | 1 | 0.03 |
| 05:00–06:00 | 0 | 0.00 | 0 | 0.00 |

# Annex 17. Mean Hourly Biting Rate of *An. minimus* s.l. from HDN in Village and Forest Fringe Sites in Stung Treng Province from December 2021 to August 2022

| Hour | HDN | | | |
|---|---|---|---|---|
| | Number Caught (annex village) (36 nights) | Biting Rate (Village) | Number Caught (forest fringe) (36 nights) | Biting Rate (forest fringe) |
| 17:00–18:00 | 0 | 0.00 | 0 | 0.00 |
| 18:00–19:00 | 0 | 0.00 | 0 | 0.00 |
| 19:00–20:00 | 1 | 0.03 | 1 | 0.03 |
| 20:00–21:00 | 0 | 0.00 | 1 | 0.03 |
| 21:00–22:00 | 2 | 0.07 | 0 | 0.00 |
| 22:00–23:00 | 0 | 0.00 | 0 | 0.00 |
| 23:00–00:00 | 0 | 0.00 | 0 | 0.00 |
| 00:00–01:00 | 0 | 0.00 | 0 | 0.00 |
| 01:00–02:00 | 0 | 0.00 | 1 | 0.03 |
| 02:00–03:00 | 0 | 0.00 | 0 | 0.00 |
| 03:00–04:00 | 0 | 0.00 | 0 | 0.00 |
| 04:00–05:00 | 0 | 0.00 | 0 | 0.00 |
| 05:00–06:00 | 0 | 0.00 | 0 | 0.00 |

# Annex 18. Adult Mosquito Collection Foci Case 1 of 4 in Mondulkiri (Royang) in May 2022

| Mosquito Species | Indoor CDC-LT | Proportion | HDN Trap | Proportion | Total | Proportion |
|---|---|---|---|---|---|---|
| *An. dirus* s.l. | 8 | 17.39% | 172 | 89.12% | 180 | 75.31% |
| *An. kochi* | 16 | 34.78% | 10 | 5.18% | 26 | 10.88% |
| *An. maculatus* s.l. | 7 | 15.22% | 2 | 1.04% | 9 | 3.77% |
| *An. vagus* | 6 | 13.04% | 0 | 0.00% | 6 | 2.51% |
| *An. minimus* s.l. | 1 | 2.17% | 2 | 1.04% | 3 | 1.26% |
| *An. barbirostris* | 2 | 4.35% | 0 | 0.00% | 2 | 0.84% |
| *An. philippinensis* | 0 | 0.00% | 2 | 1.04% | 2 | 0.84% |
| *An. indefinitus* | 1 | 2.17% | 0 | 0.00% | 1 | 0.42% |
| *An. nivipes* | 1 | 2.17% | 0 | 0.00% | 1 | 0.42% |
| *An. varuna* | 1 | 2.17% | 0 | 0.00% | 1 | 0.42% |
| Other *Anopheles* | 3 | 6.52% | 5 | 2.59% | 8 | 3.35% |
| **Total** | **46** | **100.00%** | **193** | **100.00%** | **239** | **100.00%** |

(Primary vectors highlighter in gray color)

# Annex 19. Adult Mosquito Collection Foci Case 2 of 4 in Mondulkiri (Ka Aun) in May 2022

| Mosquito Species | Indoor CDC-LT | Proportion | HDN Trap | Proportion | Total | Proportion |
|---|---|---|---|---|---|---|
| *An. philippinensis* | 5 | 41.67% | 0 | 0.00% | 5 | 38.46% |
| *An. dirus* s.l. | 3 | 25.00% | 0 | 0.00% | 3 | 23.08% |
| *An. barbirostris* | 1 | 8.33% | 0 | 0.00% | 1 | 7.69% |
| *An. nivipes* | 1 | 8.33% | 0 | 0.00% | 1 | 7.69% |
| *An. tessellatus* | 1 | 8.33% | 0 | 0.00% | 1 | 7.69% |
| *An. vagus* | 0 | 0.00% | 1 | 100.00% | 1 | 7.69% |
| Other *Anopheles* | 1 | 8.33% | 0 | 0.00% | 1 | 7.69% |
| **Total** | **12** | **100.00%** | **1** | **100.00%** | **13** | **100.00%** |

(Primary vectors highlighter in gray color)

# ANNEX 20. ADULT MOSQUITO COLLECTION FOCI CASE 3 OF 4 IN MONDULKIRI (TRANG KET) IN JULY 2022

| Mosquito Species | Indoor CDC-LT | Proportion | HDN Trap | Proportion | Total | Proportion |
|---|---|---|---|---|---|---|
| *An. philippinensis* | 3 | 14.29% | 36 | 37.50% | 39 | 33.33% |
| *An. kochi* | 3 | 14.29% | 20 | 20.83% | 23 | 19.66% |
| *An. nivipes* | 0 | 0.00% | 19 | 19.79% | 19 | 16.24% |
| *An. maculatus* s.l. | 2 | 9.52% | 11 | 11.46% | 13 | 11.11% |
| *An. dirus* s.l. | 7 | 33.33% | 4 | 4.17% | 11 | 9.40% |
| *An. vagus* | 3 | 14.29% | 1 | 1.04% | 4 | 3.42% |
| *An. indefinitus* | 1 | 4.76% | 2 | 2.08% | 3 | 2.56% |
| *An. barbirostris* | 0 | 0.00% | 1 | 1.04% | 1 | 0.85% |
| Other *Anopheles* | 2 | 9.52% | 2 | 2.08% | 4 | 3.42% |
| **Total** | **21** | **100.00%** | **96** | **100.00%** | **117** | **100.00%** |

(Primary vectors highlighter in grey color)

# ANNEX 21. ADULT MOSQUITO COLLECTION FOCI CASE 1 OF 2 IN STUNG TRENG (CHANTU KRAO) IN JULY 2022

| Mosquito Species | Indoor CDC-LT | Proportion | HDN trap | Proportion | Total | Proportion |
|---|---|---|---|---|---|---|
| *An. dirus* s.l. | 2 | 50.00% | 1 | 33.33% | 3 | 42.86% |
| *An. tessellatus* | 2 | 50.00% | 1 | 33.33% | 3 | 42.86% |
| Other *Anopheles* | 0 | 0.00% | 1 | 33.33% | 1 | 14.29% |
| **Total** | **4** | **100.00%** | **3** | **100.00%** | **7** | **100.00%** |

(Primary vectors highlighter in grey color)

# Annex 22. Adult Mosquito Collection Foci Case 4 of 4 in Mondulkiri (Pu Tuet) in September 2022

| Mosquito Species | Indoor CDC-LT | Proportion | CDN Trap | Proportion | HDN Trap | Proportion | Total | Proportion |
|---|---|---|---|---|---|---|---|---|
| *An. sinensis* | 0 | 0.00% | 124 | 14.04% | 4 | 33.33% | 128 | 14.29% |
| *An. peditaeniatus* | 0 | 0.00% | 91 | 10.31% | 0 | 0.00% | 91 | 10.16% |
| *An. indefinitus* | 0 | 0.00% | 80 | 9.06% | 2 | 16.67% | 82 | 9.15% |
| *An. splendidus* | 0 | 0.00% | 75 | 8.49% | 1 | 8.33% | 76 | 8.48% |
| *An. vagus* | 1 | 100.00% | 68 | 7.70% | 0 | 0.00% | 69 | 7.70% |
| *An. aconitus* | 0 | 0.00% | 63 | 7.13% | 1 | 8.33% | 64 | 7.14% |
| *An. crawfordi* | 0 | 0.00% | 57 | 6.46% | 2 | 16.67% | 59 | 6.58% |
| *An. kochi* | 0 | 0.00% | 55 | 6.23% | 1 | 8.33% | 56 | 6.25% |
| *An. philippinensis* | 0 | 0.00% | 29 | 3.28% | 0 | 0.00% | 29 | 3.24% |
| *An. campestris* | 0 | 0.00% | 16 | 1.81% | 0 | 0.00% | 16 | 1.79% |
| *An. nigerrimus* | 0 | 0.00% | 13 | 1.47% | 0 | 0.00% | 13 | 1.45% |
| *An. jamesii* | 0 | 0.00% | 8 | 0.91% | 0 | 0.00% | 8 | 0.89% |
| *An. maculatus* s.l. | 0 | 0.00% | 3 | 0.34% | 0 | 0.00% | 3 | 0.33% |
| *An. dirus* s.l. | 0 | 0.00% | 1 | 0.11% | 1 | 8.33% | 2 | 0.22% |
| *An. minimus* s.l. | 0 | 0.00% | 2 | 0.23% | 0 | 0.00% | 2 | 0.22% |
| *An. barbirostris* | 0 | 0.00% | 1 | 0.11% | 0 | 0.00% | 1 | 0.11% |
| *An. nivipes* | 0 | 0.00% | 1 | 0.11% | 0 | 0.00% | 1 | 0.11% |

| Mosquito Species | Indoor CDC-LT | Proportion | CDN Trap | Proportion | HDN Trap | Proportion | Total | Proportion |
|---|---|---|---|---|---|---|---|---|
| *An. argyropus* | 0 | 0.00% | 1 | 0.11% | 0 | 0.00% | 1 | 0.11% |
| Other *Anopheles* | 0 | 0.00% | 195 | 22.08% | 0 | 0.00% | 195 | 21.76% |
| **Total** | **1** | **100.00%** | **883** | **100.00%** | **12** | **100.00%** | **896** | **100.00%** |

(Primary vectors highlighter in grey color)

# Annex 23. Adult Mosquito Collection Foci Case 2 of 2 in Stung Treng (Ou Lang) in March 2023

| Mosquito Species | Indoor CDC-LT | Proportion | CDN Trap | Proportion | HDN Trap | Proportion | Total | Proportion |
|---|---|---|---|---|---|---|---|---|
| *An. vagus* | 0 | 0.0% | 3 | 50.00% | 0 | 0.00% | 3 | 42.86% |
| *An. campestris* | 0 | 0.0% | 1 | 16.67% | 0 | 0.00% | 1 | 14.29% |
| *An. dirus* s.l. | 0 | 0.0% | 0 | 0.00% | 1 | 100.00% | 1 | 14.29% |
| *An. maculatus* s.l. | 0 | 0.0% | 1 | 16.67% | 0 | 0.00% | 1 | 14.29% |
| *An. minimus* s.l. | 0 | 0.0% | 1 | 16.67% | 0 | 0.00% | 1 | 14.29% |
| **Total** | **0** | **0** | **6** | **100.00%** | **1** | **100.00%** | **7** | **100.00%** |

(Primary vectors highlighter in grey color)

# Annex 24. Trap wise Anopheles Primary Vectors Collection Details from Mondulkiri and Stung Treng Provinces during Six Foci Investigations by VectorLink Cambodia

| Type of Traps and Mosquito Collection Details | Foci 1, Royang, Mondulkiri, May 22 | Foci 2, Ka Aun, Mondulkiri, May 22 | Foci 3, Trang Ket, Mondulkiri, July 22 | Foci 4, Chantu Krao Thom, Stung Treng, July 22 | Foci 5, Pu Tuet, Mondulkiri, September 22 | Foci 6, Ou Lang, Stung Treng, March 23 |
|---|---|---|---|---|---|---|
| **HDN (outdoor)** | | | | | | |
| *An. minimus* s.l. | 2 | 0 | 0 | 0 | 0 | 0 |
| *An. maculatus* s.l. | 2 | 0 | 11 | 0 | 0 | 0 |
| *An. dirus* s.l. | 172 | 0 | 4 | 1 | 1 | 1 |
| Other *Anopheles* | 17 | 1 | 81 | 2 | 11 | 0 |
| **Sub total** | **193** | **1** | **96** | **3** | **12** | **1** |
| **CDN (outdoor)** | | | | | | |
| *An. minimus* s.l. | - | - | - | - | 2 | 1 |
| *An. maculatus* s.l. | - | - | - | - | 3 | 1 |
| *An. dirus* s.l. | - | - | - | - | 1 | 0 |
| Other *Anopheles* | - | - | - | - | 877 | 4 |
| **Sub total** | **-** | **-** | **-** | **-** | **883** | **6** |

| Type of Traps and Mosquito Collection Details | Foci 1, Royang, Mondulkiri, May 22 | Foci 2, Ka Aun, Mondulkiri, May 22 | Foci 3, Trang Ket, Mondulkiri, July 22 | Foci 4, Chantu Krao Thom, Stung Treng, July 22 | Foci 5, Pu Tuet, Mondulkiri, September 22 | Foci 6, Ou Lang, Stung Treng, March 23 |
|---|---|---|---|---|---|---|
| **CDC LT (indoor)** | | | | | | |
| *An. minimus* s.l. | 1 | 0 | 0 | 0 | 0 | 0 |
| *An. maculatus* s.l. | 7 | 0 | 2 | 0 | 0 | 0 |
| *An. dirus* s.l. | 8 | 3 | 7 | 2 | 0 | 0 |
| Other *Anopheles* | 30 | 9 | 12 | 2 | 1 | 0 |
| **Sub total** | **46** | **12** | **21** | **4** | **1** | **0** |
| **Grand Total** | **239** | **13** | **117** | **7** | **896** | **7** |

# Annex 25. Number of Anopheles Primary Vectors Tested For Elisa from Mondulkiri and Stung Treng Provinces during Six Foci Investigations by VectorLink Cambodia

| Place of Collection | Morphological Identification | Total Screened | Molecular Identification | Number Found Positive | Sporozoite Rate |
|---|---|---|---|---|---|
| Mondulkiri (Royang) | *An. dirus* s.l. | 180 | *An. dirus* s.s. | 1 (*P.v210*) | 0.56 |
| | *An. maculatus* s.l. | 9 | | 0 | 0 |
| | *An. minimus* s.l. | 3 | | 0 | 0 |
| Mondulkiri (Pu Til) | *An. dirus* s.l. | 1 | | 0 | 0 |
| | *An. maculatus* s.l. | 3 | | 0 | 0 |
| | *An. minimus* s.l. | 1 | | 0 | 0 |
| Mondulkiri (Ka Aun) | *An. dirus* s.l. | 3 | | 0 | 0 |
| Mondulkiri (Trang Ket) | *An. dirus* s.l. | 11 | | 0 | 0 |
| | *An. maculatus* s.l. | 14 | | 0 | 0 |
| Stung Treng (Ou Lang) | *An. dirus* s.l. | 1 | | 0 | 0 |
| | *An. maculatus* s.l. | 1 | | 0 | 0 |
| | *An. minimus* s.l. | 1 | | 0 | 0 |
| Stung Treng (Chantu Krao) | *An. dirus* s.l. | 3 | | 0 | 0 |
| **Total** | | **1** | | **1** | |

# ANNEX 26. NUMBER OF *ANOPHELES* SPECIES SCREENED FOR MOLECULAR IDENTIFICATION FROM MONDULKIRI AND STUNG TRENG DURING SIX FOCI INVESTIGATIONS BY VECTORLINK CAMBODIA

| Morphological Identification | Molecular Identification | Foci_1 | Foci_2 | Foci_3 | Foci_4 | Foci_5 | Foci_6 | Total |
|---|---|---|---|---|---|---|---|---|
| | | Royang Mondulkiri | Ka Aun Mondulkiri | Trang Ket Mondulkiri | Chantu Krao Stung Treng | Pu Tuet Mondulkiri | Ou Lang Stung Treng | |
| | | May-2022 | May-2022 | July-2022 | July-2022 | Sept.-2022 | March-2023 | |
| *An. dirus* s.l. (n=199) | *An. dirus* s.s. | 148 | 3 | 10 | 2 | 1 | 1 | 165 |
| | *An. scanloni* | | | | | | | - |
| | *An. cracens* | | | | | | | - |
| | *An. nemophilous* | | | | | | | - |
| | *An. baimaii* | | | | | | | - |
| | Unamplified | 32 | | 1 | 1 | | | 34 |

| Morphological Identification | Molecular Identification | Foci_1 | Foci_2 | Foci_3 | Foci_4 | Foci_5 | Foci_6 | Total |
|---|---|---|---|---|---|---|---|---|
| | | Royang Mondulkiri | Ka Aun Mondulkiri | Trang Ket Mondulkiri | Chantu Krao Stung Treng | Pu Tuet Mondulkiri | Ou Lang Stung Treng | |
| | | May-2022 | May-2022 | July-2022 | July-2022 | Sept.-2022 | March-2023 | |
| *An. minimus* s.l. (n=5) | *An. minimus* s.s. | 1 | | | | | 1 | 2 |
| | *An. harrisoni* | | | | | | | - |
| | *An. aconitus* | | | | | 1 | | 1 |
| | *An. varuna* | | | | | | | - |
| | *An. pampanai* | | | | | | | - |
| | Unamplified | 2 | | | | | | 2 |
| *An. maculatus* s.l. (n=27) | *An. maculatus* s.s. | | | | | | | |
| | *An. pseudowillmori* | | | | | | | |
| | *An. sawadwongporni* | 2 | | 9 | | 2 | | 13 |
| | *An. rampae* (form K) | 6 | | 4 | | | 1 | 11 |
| | *An. dravidicus* | | | | | | | |
| | Unamplified | 1 | | 1 | | 1 | | 3 |