# DIVERSITY, SEASONAL ABUNDANCE AND BITING ACTIVITY OF *ANOPHELES* SPECIES IN RELATION TO CLIMATIC FACTORS IN NORTHEASTERN THAILAND

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**Abstract.** An outbreak of malaria recently occurred in Ubon Ratchathani Province, northeastern Thailand where a high prevalence of artemisinin-resistant *Plasmodium falciparum* has been reported. In this study, we investigated diversity, seasonal abundance and degree of outdoor biting activity of *Anopheles* species collected in buffalo-baited traps from January 2014 to December 2015. Of 11,765 *Anopheles* females belonging to 14 species collected, the four most prevalent taxa were species of the *An. hyrcanus* group (58.89%), the *An. barbirostris* complex (29.83%), *An. nivipes* (5.41%) and *An. philippinensis* (4.30%). The identities of nine species, namely, *An. dirus*, *An. dissidens*, *An. minimus*, *An. nigerrimus*, *An. nitidus*, *An. nivipes*, *An. peditaeniatus*, *An. philippinensis*, and *An. rampae*, were confirmed molecularly using an allele-specific (AS)-PCR assay based on sequences of the second internal transcribed spacer (ITS2) region of rDNA and mitochondrial cytochrome oxidase I (*COI*) gene. The density of *An. barbirostris* s.l. showed a major peak during the rainy and cool-dry seasons, whereas members of the *An. hyrcanus* group were most abundant in the rainy season. A significant difference in the time of biting activity, based on mean numbers of captured females, was observed between members of the *An. hyrcanus* group, *An. barbirostris* s.l. and *An. nivipes*. In addition, an increase in rainfall and relative humidity influenced densities of species of the *An. hyrcanus* group and the *An. barbirostris* complex. These data lend support to the need for further studies on vectorial status of the *Anopheles* taxa in malaria endemic areas.

**Keywords:** *Anopheles*, allele-specific PCR, *COI*, malaria, species diversity

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This paper is dedicated to the late Professor Wej Choochote.

## INTRODUCTION

Malaria still remains a health problem in Thailand. The highest incidence of malaria in the country ($n$ = 12,163) was reported between 2014 and 2015 in Ubon Ratchathani Province, located along the Thai-Cambodia and Thai-Lao PDR borders in northeastern Thailand (Bureau of Vector Borne Diseases, 2014). It is noteworthy that the number of malaria cases in this province increased from 618 in 2013 to 8,836 in 2014 (Bureau of Vector Borne Diseases, 2014). Illegal cutting of Siamese rosewood (*Dalbergia cochinchinensis*) in forests during 2013 to 2014 is a potential contribution to the outbreak of malaria in the region (Lyttleton, 2016).

In Thailand, the most important malaria vectors are members of the sibling species complexes or groups of morphologically similar species, the primary vectors being *An. baimaii* and *An. dirus* (species of the *An. dirus* complex), *An. minimus* (a species of the *An. minimus* complex) and *An. maculatus* (a species of the *An. maculatus* group) (Taai *et al*, 2017). Other members of the *An. maculatus* group (*An. pseudowillmori* and *An. sawadwongporni*), members of the *An. barbirostris* complex and a member of the *An. sundaicus* complex (*An. epiroticus*), as well as *An. aconitus* (a close relative of *An. minimus*), have been incriminated as secondary vectors in the country (Tainchum *et al*, 2015).

However, sibling and closely related species are isomorphic or have overlapping morphological features, and are therefore easily and often misidentified. Consequently, integrated morphological and molecular means of identification have been used in many studies to ensure reliable species identification, *viz*. studies on the biting activity and host preferences of members of the *An. dirus* complex, *An. minimus* complex and the *An. maculatus* group in two malaria endemic areas of northwestern Thailand (Tainchum *et al*, 2014); the *An. minimus* and *An. dirus* complexes in a malaria endemic region of western Thailand (Tananchai *et al*, 2012; Tisgratog *et al*, 2012); and *An. epiroticus* of the *An. sundaicus* complex in a malaria endemic region of eastern Thailand (Sumruayphol *et al*, 2010; Ritthison *et al*, 2014).

It has long been realized that knowledge of the bionomics of individual vector species of malarial protozoa is necessary for designing effective control strategies against target species (Tisgratog *et al*, 2012). However, information on the bionomics of *Anopheles* mosquitoes in malaria endemic area of Ubon Ratchathani Province is lacking. Thus, the aim of this study was to determine the diversity, seasonal abundance and the degree of outdoor biting activity of *Anopheles* species during a two-year study in the malaria endemic Na Chaluai District, Ubon Ratchathani Province using a molecular identification assay for reliable species identification.

## MATERIALS AND METHODS

### Study site

Anopheline mosquitoes were collected at a fixed location (wood hut) located in a forest fringe near Phu Chong Na Yoi National Park (14.43858° N, 105.23485° E), Ban Kaeng Ruang Village, Na Chaluai District, Ubon Ratchathani Province (Fig 1). This study area was selected based on the high endemicity of malaria (4,103 cases in 2014 and 2015) from data of the Bureau of Vector Borne Diseases, Ministry of Public Health, Thailand. The study site is surrounded by forest, small rice fields, and cassava and rubber plantations, and is approximately 200 m above sea level.

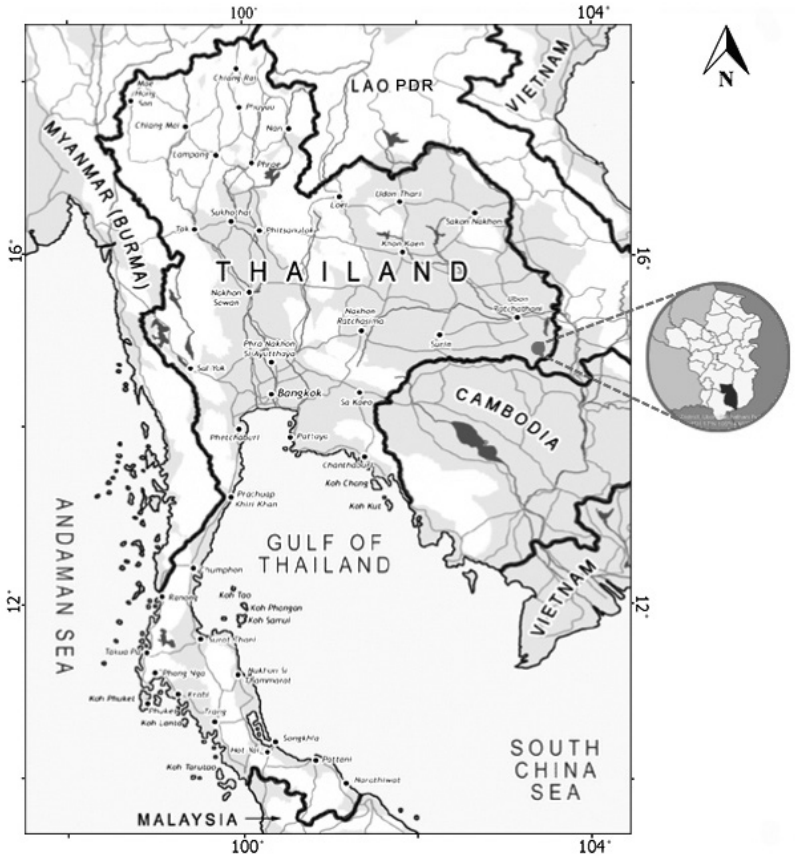


Fig 1–Study area for collection of adult female anopheline mosquitoes in Na Chaluai District, Ubon Ratchathani Province, Thailand, January 2014 - December 2015.

**Mosquito collection**

Outdoor buffalo-baited collections of adult female anophelines were made on four consecutive nights each month from 06:00 PM to 06:00 AM over a two-year period of study (January 2014 and December 2015). A buffalo was tethered and enclosed by a net with a 30 cm gap above ground level (Tananchai *et al*, 2012), and was exposed to mosquitoes entering the net for 50 minutes each hour (Junkum *et al*, 2007). All *Anopheles* mosquitoes resting on the inside walls of the net after having bitten the buffalo were collected using an aspirator during 10 minutes each hour.

Biting times are classified as early evening (06:00-09:00 PM), late night (09:00-12:00 PM), pre-dawn (12:00 PM-03:00 AM) and dawn (03:00-06:00 AM) (Tisgratog *et al*, 2012). Three seasons of the year, based on temperature and rainfall data obtained from the Climatology Division (code station 407021), Meteorological Department, Ministry of Information and Communication Technology, Bangkok, Thailand, located in Na Chaluai District, are the hot-dry (March to May), wet (June to October) and cool-dry (November to February) seasons. Ambient air temperature and relative humidity were recorded each hour using a DHT-1 digital hygro-thermometer (Daeyoon Scale Industrial, Seoul, South Korea). Captured mosquitoes were transported at 25°C to the laboratory at the Office of Disease Prevention and Control Region 10, Ubon Ratchathani Province for species identification.

**Morphological species identification**

Each female mosquito was identified to species using available illustrated keys (Reid, 1968; Harrison and Scanlon, 1975; Rattanarithikul *et al*, 2006; Somboon and Rattanarithikul, 2013).

Table 1
Primers used for identification of *Anopheles* species based on ITS2 and *COI* sequences.

| *Anopheles* taxon | Primer | Sequences (5′-3′) | Amplicon size (bp) |
|---|---|---|---|
| ITS2 sequence | | | |
| *An. dirus* | | | 562 |
| Forward primer | D-U | CGCCGGGGCCGAGGTGG | |
| Species-specific reverse primer | D-AC | CACAGCGACTCCACACG | |
| *An. minimus* | | | 310 |
| Universal forward primer | ITS2A | TGTGAACTGCAGGACACAT | |
| Species-specific reverse primer | MIA | CCCGTGCGACTTGACGA | |
| *An. rampae* | | | 301 |
| Forward primer | 5.8F | ATCACTCGGCTCGTGGATCG | |
| Species-specific reverse primer | K | TTCATCGCTCGCCCTTACAA | |
| *An. nivipes* | | | 245 |
| Forward primer | 5.8S | TGTGAACTGCAGGACACATG | |
| Reverse primer | NIV | CATGTACCTCACGATACATGTA | |
| *An. philippinensis* | | | 163 |
| Forward primer | 5.8S | TGTGAACTGCAGGACACATG | |
| Reverse primer | PHI | GCACGCCATTATGCGACAAAC | |
| *COI* sequence | | | 658 |
| Universal forward primer | LCO1490 | GGTCAACAAATCATAAAGATATTGG | |
| Universal reverse primer | HCO2198 | TAAACTTCAGGGTGACCAAAAAATCA | |

**Molecular-based species identification**

To confirm the morphological identifications, cryptic *Anopheles* species of groups or complexes were further identified using a PCR-based assay (Walton *et al*, 1999, 2007; Saeung *et al*, 2007, 2008; Suwannamit *et al*, 2009; Thongsahuan *et al*, 2009; Wijit *et al*, 2013). Briefly, genomic DNA was extracted from the whole body or wings and legs of individual mosquitoes using the PureLink® Genomic DNA Kit (Invitrogen, Carlsbad, CA). AS-PCR was used to identify species of the *An. dirus* complex (Walton *et al*, 1999), the *An. minimus* complex (Garros *et al*, 2004), *An. nivipes*/*An. philippinensis*, and members of the *An. maculatus* group (Walton *et al*, 2007). Mitochondrial cytochrome c oxidase subunit I gene (*COI*)-based DNA barcoding was used to identify members of the *An. hyrcanus* group (Wijit *et al*, 2013) and sibling species of the *An. barbirostris* complex (Saeung *et al*, 2007, 2008; Suwannamit *et al*, 2009; Thongsahuan *et al*, 2009). Primers and amplicon sizes are listed in Table 1. Amplicons were analysed by 1.5% agarose gel-electrophoresis, stained with SYBR® Safe DNA gel stain (Invitrogen, Carlsbad, CA) and directly sequenced (Macrogen, Seoul, South Korea). The sequences obtained were deposited to the DDBJ/EMBL/GenBank nucleotide sequence database under accession numbers LC333225-LC333267, and compared with sequences available in GenBank (*An. dirus*: accession numbers KP298431 and KP298432; *An. dissidens*: accession numbers AB971323 and AB971324; *An.*

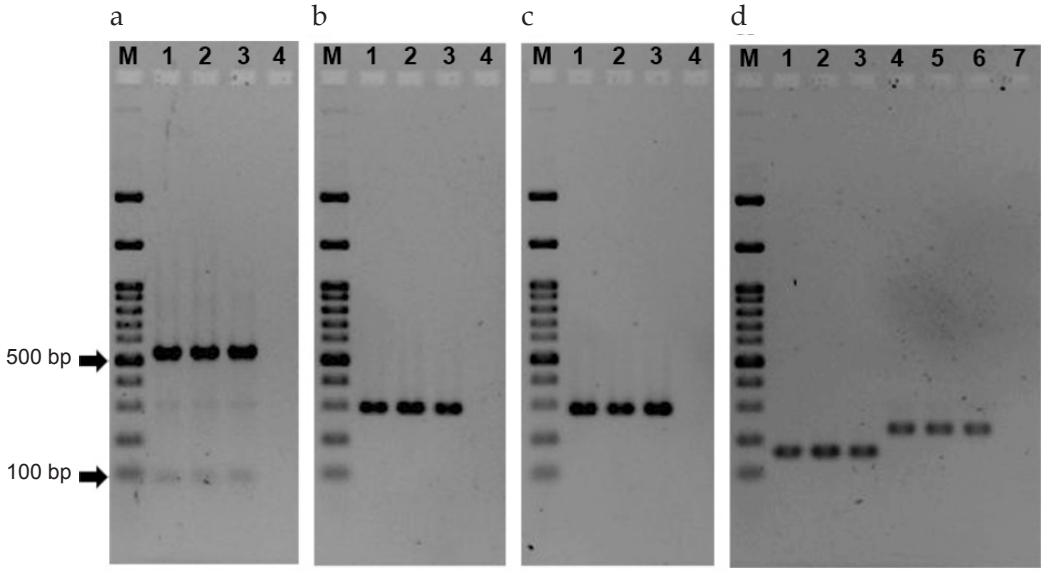


Fig 2–Allele-specific PCR assay for identifying (a) *An. dirus* (562 bp), (b) *An. minimus* (310 bp), (c) *An. rampae* (301 bp), and (d) *An. philippinensis* (163 bp). (a), (b) and (c): Lanes 1-3, samples; lane 4, negative control. (d): Lanes 1-3, samples; lanes 4-6, *An. nivipes* (245 bp); lane 7, negative control. Lane M, 100 bp size markers.

*minimus*: accession numbers FN646403 and KP298408, *An. nigerrimus*: accession numbers AB778789 and AB778798, *An. nitidus*: accession numbers AB781762 and AB781766, *An. nivipes*: accession numbers FJ526621 and EU919722, *An. peditaeniatus*: accession numbers AB781772 and AB781776, *An. philippinensis*: accession numbers DQ319187 and JN654434, and *An. rampae* accession numbers AY803340 and AY803341) using Basic Local Alignment Search Tool (BLAST), (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

**Data analysis**

Comparisons of mosquito capture data were analyzed using ANOVA. A post-hoc Tukey's honest significant difference (HSD) test was used for multiple comparisons of means. The level of significance is set at 5% ($p < 0.05$). Pearson's correlation coefficients ($R$) were computed to determine the association between mosquito abundance and climatic variables (rainfall, relative humidity and temperature). All data were analyzed using IBM SPSS statistics, version 22 for Windows (IBM, Armonk, NY).

## RESULTS

**Mosquito species composition**

Based on morphological identification, the 11,765 *Anopheles* females collected during the two-year study belonged to 12 species/groups of the subgenus *Cellia* and two groups of the subgenus *Anopheles* (Table 2). The four most abundant taxa were members of the *An. hyrcanus* group (58.89%), the *An. barbirostris* complex (29.83%), *An. nivipes* (5.41%) and *An. philippinensis* (4.30%). Members of the three primary malaria vector taxa in Thailand, the *An. dirus* complex, the *An. minimus* complex and the *An. maculatus* group,

Table 2
Numbers of *Anopheles* mosquitoes collected from buffalo-bait traps in Na Chaluai District, Ubon Ratchathani Province, Thailand, January 2014 - December 2015.

| *Anopheles* taxon | 2014 | 2015 | Total | % |
|---|---|---|---|---|
| *An. barbirostris* s.l. | 1,573 | 1,937 | 3,510 | 29.83 |
| *An. hyrcanus* group | 4,353 | 2,575 | 6,928 | 58.89 |
| *An. aconitus* | 2 | 0 | 2 | 0.02 |
| *An. annularis* | 18 | 1 | 19 | 0.16 |
| *An. jamesii* | 8 | 0 | 8 | 0.07 |
| *An. karwari* | 33 | 5 | 38 | 0.32 |
| *An. kochi* | 13 | 16 | 29 | 0.25 |
| *An. nivipes* | 313 | 323 | 636 | 5.41 |
| *An. philippinensis* | 275 | 231 | 506 | 4.30 |
| *An. tessellatus* | 1 | 0 | 1 | 0.01 |
| *An. vagus* | 5 | 8 | 13 | 0.11 |
| *An. minimus* s.l. | 6 | 4 | 10 | 0.08 |
| *An. maculatus* group | 42 | 17 | 59 | 0.50 |
| *An. dirus* s.l. | 4 | 2 | 6 | 0.05 |
| Total | 6,646 | 5,119 | 11,765 | 100 |

Table 3
Molecular identification of *Anopheles* species.

| *Anopheles* taxon[a] | Molecular identification | Number of specimens |
|---|---|---|
| *An. dirus* s.l. | *An. dirus* | 5 |
| *An. minimus* s.l. | *An. minimus* | 8 |
| *An. maculatus* group | *An. rampae* | 15 |
| *An. barbirostris* s.l. | *An. dissidens* | 13 |
| *An. hyrcanus* group | *An. peditaeniatus* | 9 |
| | *An. nitidus* | 17 |
| | *An. nigerrimus* | 1 |
| *An. nivipes* | *An. nivipes* | 19 |
| *An. philippinensis* | *An. philippinensis* | 27 |
| Total | | 114 |

[a]Based on morphological identification.

comprised <1% of the total collection. Identifications of representative specimens ($n$ = 114) belonging to three species complexes (*An. barbirostris*, *An. dirus* and *An. minimus* complexes) and two groups (*An. hyrcanus* and *An. maculatus* groups), as well as *An. nivipes*/*An. philippinensis* were confirmed by AS-PCR and *COI* barcoding assays (Fig 2, Table 3).

**Seasonal abundance of the most prevalent *Anopheles* taxa**

Overall there is no significant difference between the mean number of mos-

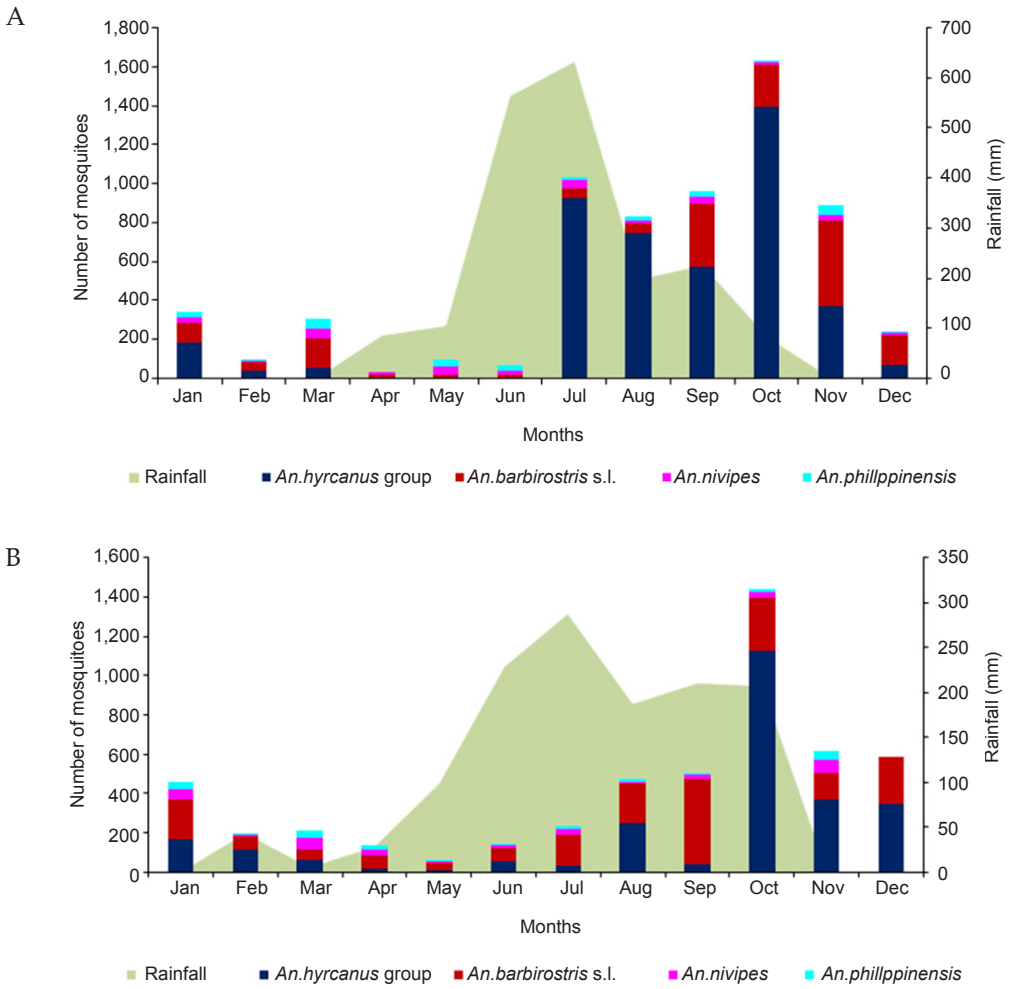


Fig 3–Monthly frequency of the four most prevalent *Anopheles* taxa collected from buffalo-baited traps in Na Chaluai District, Ubon Ratchathani Province, Thailand, January 2014 - December 2014 (A) and January 2015 - December 2015 (B). Shading represents rainfall (mm).

quitoes collected in 2014 and 2015 (Table 4). However, there is a significant difference between the number of mosquitoes of the *An. hyrcanus* group and of the *An. barbirostris* complex captured in the hot-dry and wet seasons (Tukey's HSD test, $p = 0.039$ and 0.002, respectively) (Table 4). Greater numbers of *An. barbirostris* s.l. were collected during the rainy and cool-dry seasons, with a distinct peak in November of 2014 (Fig 3A) and September 2015 (Fig 3B). Members of the *An. hyrcanus* group were more abundant during the rainy season, with peaks obtained in October of both years (Fig 3). High densities of *An. nivipes* and *An. philippinensis* were recorded during the hot and cool-dry seasons (Fig 3).

**Host-feeding patterns of the most prevalent *Anopheles* taxa**

Outdoor biting activity of the four

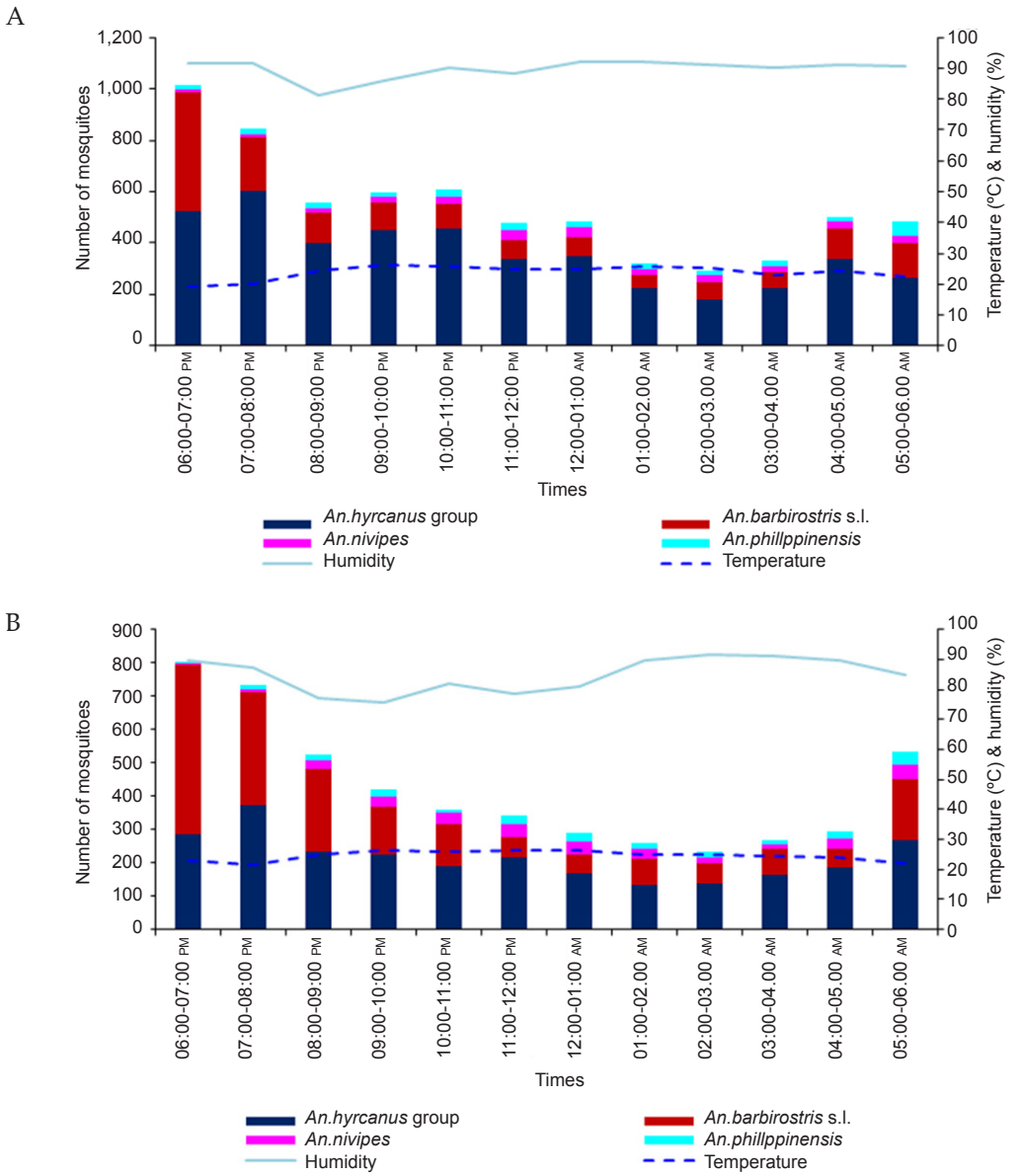


Fig 4–Hourly outdoor biting activity of the four most prevalent *Anopheles* taxa collected from buffalo-baited traps in Na Chaluai District, Ubon Ratchathani Province, Thailand, January 2014 - December 2014 (A) and January 2015 - December 2015 (B).

most prevalent taxa feeding on buffalo bait during the two-year study revealed greatest biting activity of *An. barbirostris* s.l., members of the *An. hyrcanus* group and *An. philippinensis* between 06:00-07:00 PM, 07:00-08:00 PM and 05:00-06:00 AM, respectively in both years (Fig 4). However, two different peaks were recorded in 2014 (11:00-12:00 PM) (Fig 4A) and 2015 (05:00-06:00 AM) (Fig 4B) for *An. nivipes*. Significant differences in the mean numbers of members of the *An. hyrcanus* group, mem-

Table 4
Comparison of numbers of the four most prevalent *Anopheles* taxa collected from buffalo-baited traps in Na Chaluai District, Ubon Ratchathani Province, Thailand, January 2014 - December 2015.

| *Anopheles* taxon | Year | | | Season | | | Post-hoc test | Time period | | | Post-hoc test |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | df | *F* | *p*-value | df | *F* | *p*-value | | df | *F* | *p*-value | |
| *An. hyrcanus* group | 1 | 0.068 | 0.795 | 2 | 3.928 | 0.036[a] | dry-wet ($p = 0.039$)[b] | 3 | 4.318 | 0.017[a] | EE-PD ($p = 0.015$)[b] |
| *An. barbirostris* s.l. | 1 | 1.204 | 0.272 | 2 | 7.672 | 0.008[a] | dry-wet ($p = 0.002$)[b] | 3 | 11.624 | 0.008[a] | EE-LN ($p = 0.001$)[b] |
| | | | | | | | | | | | EE-PD ($p < 0.001$)[b] |
| | | | | | | | | | | | EE-D ($p = 0.001$)[b] |
| *An. nivipes* | 1 | 0.041 | 0.84 | 2 | 1.356 | 0.279 | | 3 | 6.182 | 0.004[a] | EE-LN ($p = 0.005$)[b] |
| | | | | | | | | | | | EE-PD ($p = 0.011$)[b] |
| | | | | | | | | | | | EE-D ($p = 0.030$)[b] |
| *An. philippinensis* | 1 | 0.188 | 0.664 | 2 | 0.287 | 0.711 | | 3 | 1.878 | 0.329 | |

Season = hot-dry, wet and cool-dry. Time period = Year = 2014 and 2015. D, dawn; EE, early evening; LN, late night; PD, pre-dawn
[a]Statistically significant difference, ANOVA. [b]Statistically significant difference, Tukey's HSD.

bers of the *An. barbirostris* complex and *An. nivipes* were found between different time periods (Table 4).

**Effects of variables on seasonal *Anopheles* abundance**

Pearson's analysis of correlation between the four most prevalent taxa and climatic variables showed a positive correlation between the number of females of the *An. hyrcanus* group and rainfall ($R = 0.640$, $p = 0.025$) in 2014 (Table 5), whereas the density of *An. barbirostris* s.l. correlated with relative humidity ($R = 0.711$, $p = 0.010$) in 2015 (Table 5).

## DISCUSSION

The emergence and geographic spread of artemisinin-resistant *P. falciparum* in the Greater Mekong sub-region (GMS) represents a serious threat to global malaria control (Imwong *et al*, 2017). Previously, Imwong *et al* (2015) reported the majority of *P. falciparum* strains responsible for the outbreak of malaria in Ubon Rachathani Province are artemisinin-resistant, as defined by the presence of certain mutations in the *PfKelch* on chromosome 13. Moreover, malaria transmission along the Thai borders with Cambodia and Lao PDR is difficult to control due to such factors as the presence of *Anopheles* vectors with changes in biting behavior, unrestricted movement of people across the borders, and immigration which might make a disproportionate contribution to the burden of regional malaria (Corbel *et al*, 2013). The anopheline vectors in the GMS primarily bite outdoors, enabling them to avoid commonly used vector control interventions, such as indoor

Table 5
Pearson's correlation coefficient (*R*) of the four most prevalent *Anopheles* taxa collected from buffalo-baited traps in Na Chaluai District, Ubon Ratchathani Province, Thailand, January 2014 - December 2015.

| *Anopheles* taxon | January 2014 - December 2014 | | | | | |
|---|---|---|---|---|---|---|
| | Rainfall | | Temperature | | Relative humidity | |
| | *R* | *p*-value | *R* | *p*-value | *R* | *p*-value |
| *An. hyrcanus* group | 0.640 | 0.025* | 0.077 | 0.811 | 0.368 | 0.239 |
| *An. barbirostris* s.l. | -0.158 | 0.623 | -0.005 | 0.987 | 0.125 | 0.698 |
| *An. nivipes* | 0.020 | 0.951 | 0.351 | 0.263 | -0.427 | 0.166 |
| *An. philippinensis* | -0.128 | 0.692 | 0.190 | 0.555 | -0.305 | 0.335 |
| | January 2015 - December 2015 | | | | | |
| *An. hyrcanus* group | -0.269 | 0.398 | -0.278 | 0.382 | 0.519 | 0.084 |
| *An. barbirostris* s.l. | 0.011 | 0.973 | -0.194 | 0.546 | 0.711 | 0.010* |
| *An. nivipes* | -0.445 | 0.147 | -0.407 | 0.189 | -0.098 | 0.761 |
| *An. philippinensis* | -0.245 | 0.442 | -0.343 | 0.275 | -0.193 | 0.549 |

residual sprays or insecticide-treated bed nets (Durnez *et al*, 2013).

Although human-landing collections provide direct evidence of anthropophagy, several alternative sampling methods, *eg*, CDC miniature light trap (Sriwichai *et al*, 2016) and cattle-baited collection (Tisgratog *et al*, 2012; St Laurent *et al*, 2016), have been used in many regions to monitor and evaluate populations of malaria vectors. In this study, we recorded the diversity of *Anopheles* taxa captured outdoors using a buffalo-baited trap in a malaria endemic area for two consecutive years. Members of the *An. hyrcanus* group, the *An. barbirostris* complex, *An. nivipes* and *An. philippinensis* were the predominate zoophilic species, which stands in contrast to very low numbers of females of the principal malaria vector taxa, *ie*, members of the *An. dirus* complex, the *An. minimus* complex and the *An. maculatus* group. This observation is in agreement with that of St Laurent *et al* (2016) in Cambodia where *An. barbirostris* s.l., *An. nivipes* and *An. philippinensis* were found to be the most dominant species, and all of the *Plasmodium*-infected mosquitoes were members of the *An. hyrcanus* group and the *An. barbirostris* complex collected in cow-baited tents. In addition, Sriwichai *et al* (2016) found that two primary malaria vector taxa, *An. minimus* s.l. and *An. maculatus* group, taken in cow-baited collections, were positive for *P. vivax* anti-circumsporozoite protein (Pv-247) antibodies using ELISA. Thus, further studies are needed to determine whether or not the *Anopheles* collected during the present study are vectors of human malaria parasites.

As sibling species are essentially isomorphic and groups of closely related species have minimal morphological distinctions, researchers have had to develop reliable molecular-based assays for species identification. For example, Walton *et al* (1999) developed an AS-PCR

assay based on ITS2 sequences to distinguish *An. dirus*, *An. cracens*, *An. scanloni*, *An. baimaii* and *An. nemophilus* of the *An. dirus* complex. Likewise, AS-PCR assay was used to distinguish *An. minimus* and *An. harrisoni*, two species of the *An. minimus* complex (Phuc *et al*, 2003; Garros *et al*, 2004; Garros *et al*, 2005a, b; Manguin *et al*, 2008). In addition, several investigators have applied *COI* barcoding for the identification of sibling species of *An. barbirostris* complex (Saeung *et al*, 2007, 2008; Suwannamit *et al*, 2009; Thongsahuan *et al*, 2009; Taai and Harbach, 2015). Furthermore, Wijit *et al* (2013) used *COI* barcode sequence for the identification of eight species of the *An. hyrcanus* group (*An. argyropus*, *An. crawfordi*, *An. nigerrimus*, *An. nitidus*, *An. paraliae*, *An. peditaeniatus*, *An. pursati* and *An. sinensis*), which are known to occur in Thailand. Thus, we used both of these methods to identify a selection of specimens collected during each time period and season.

The abundance of *An. barbirostris* s.l. collected was comparable with the abundance of this taxon reported by Sriwichai *et al* (2016) who captured this species only in the wet season, with a peak density in September. Similarly, St Laurent *et al* (2016) recorded, based on cow-baited collection, peak biting activity of *An. barbirostris* s.l. and *An. nivipes* between 06:00 PM and 08:00 PM and 05:00 and 06:00 AM, respectively. As with the present study, Semuel *et al* (2016) reported that *An. barbirostris* s.l. reaches peak biting activity in early evening (08:00 PM).

The seasonal abundance of members of the *An. hyrcanus* group and the *An. barbirostris* complex appeared to be influenced by rainfall and relative humidity, respectively. Similarly, Bashar and Tuno (2014) demonstrated that rainfall and relative humidity are the most important factors influencing the densities of *An. baimaii* and *An. willmori* in Kumari, Bangladesh. However, no significant association between mosquito densities and temperature was found in the present study.

In conclusion, this study is the first detailed investigation of species diversity, seasonal abundance and outdoor biting activity of *Anopheles* mosquitoes using buffalo-baited collections in a malaria endemic region of Ubon Ratchathani Province, northeastern Thailand. Members of the *An. hyrcanus* group, the *An. barbirostris* complex, *An. nivipes* and *An. philippinensis* were the most prevalent taxa collected. High rainfall and relative humidity have an impact on population densities of species of the *An. hyrcanus* group and the *An. barbirostris* complex. The findings of this study should be useful for future investigations into the potential role these *Anopheles* in malaria transmission in Ubon Ratchathani Province.

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## CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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