New records and updated checklist of mosquitoes (Diptera: Culicidae) from Lao People's Democratic Republic, with special emphasis on adult and larval surveillance in Khammuane Province

Maysa T. Motoki[1,2,✉], Khamsing Vongphayloth[1], Leopoldo M. Rueda[2], Elliott F. Miot[1,3,4,5], Alexandra Hiscox[6], Jeffrey C. Hertz[7], and Paul T. Brey[1]

[1]*Medical Entomology and Vector-Borne Disease Unit, Institut Pasteur du Laos, Samsenthai Rd., Ban Kao-gnot, P.O. Box 3560, Vientiane, Lao PDR, maysatm@gmail.com*
[2]*Walter Reed Biosystematics Unit, Entomology Department, Smithsonian Institution, MSC MRC 514, Suitland, MD, U.S.A.*
[3]*Université Pierre et Marie Curie, Cellule Pasteur UPMC, Paris, France*
[4]*Insect-Virus Interactions Group, Department of Genomes and Genetics, Institut Pasteur, Paris, France*
[5]*Centre National de la Recherche Scientifique, Unité Mixte de Recherche 2000, Paris, France*
[6]Laboratory of Entomology, Wageningen University and Research, Wageningen, The Netherlands
[7]U.S. Naval Medical Research Unit 2, Sembawang, Singapore

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ABSTRACT: A list of mosquitoes from the Nakai Nam Theun National Protected Area along the Nam Theun, Nam Mon, Nam Noy, and Nam On rivers, Nakai District, Khammuane Province, Lao People's Democratic Republic (Lao PDR) is presented. Fifty-four mosquito taxa were identified, including 15 new records in the Lao PDR. A fragment of the mtDNA cytochrome c oxidase subunit I (*COI*) gene, barcode region, was generated for 34 specimens, and together with four specimens already published, it represented 23 species in eight genera. In addition, an updated checklist of 170 mosquito taxa from Lao PDR is provided based on field collections from Khammuane Province, the literature, and specimens deposited in the Smithsonian Institution, National Museum of Natural History (SI-NMNH), Washington, DC, U.S.A. This paper provides additional information about the biodiversity of mosquito fauna in Lao PDR. ***Journal of Vector Ecology* 44 (1): 76-88. 2019**

Keyword Index: Culicidae, mosquito surveillance, DNA barcode, Lao PDR, Khammuane Province, mosquito species list.

INTRODUCTION

Cambodia, China, Myanmar, Thailand, Vietnam, and Lao People's Democratic Republic (Lao PDR) constitute the Greater Mekong Sub-region (GMS), where diseases caused by mosquito-borne pathogens remain a significant source of morbidity (Jones et al. 2008, Hii and Rueda 2011). Malaria and dengue are the most important public health challenges in the GMS region (Hewitt et al. 2013, Suwonkerd et al. 2013, Undurraga et al. 2013, WHO 2016), while other mosquito-borne infections like chikungunya, Zika, and Japanese encephalitis remain a persistent threat (Hotez et al. 2015). Furthermore, the human population movement between Africa and China has resulted in an increased risk of yellow fever cases in China (Ling et al. 2016).

In Lao PDR, the major diseases of the region are transmitted by known species, which act as a primary vector in the surrounding southeast countries. *Aedes aegypti* (Linnaeus), the primary vector of dengue, chikungunya, and Zika viruses (WHO 2009, Lao et al. 2014) is the main vector for all four serotypes of dengue flaviviruses (DENV1-4) in Lao PDR (Khampapongpane et al. 2014, Somlor et al. 2017). *Anopheles dirus* Peyton and Harrison, *An. maculatus* Theobald and *An. minimus* Theobald are the most important vectors of malaria (Pholsena 1992, Meek 1995, Toma et al. 2002, Vythilingam et al. 2005, Jorgensen et al. 2010, Hii and Rueda 2013, Suwonkerd et al. 2013). Despite some studies that identified *Plasmodium* in the mosquito salivary glands (Toma et al. 2002), little is known about the vector status, distribution, and ecology of not only the anopheline, but of most species of culicidae in Lao PDR.

Nakai Nam Theun National Protected Area (NNT NPA), known as the Watershed Management and Protection Authority (WMPA), is Lao PDR's second largest national protected area in Nakai District, Khammuane Province. It is recognized as an important biodiversity site in Southeast Asia and globally, containing mammals, birds, reptiles, amphibians (WCS 2018), and insects, including mosquitoes. It is also the home of rare or newly discovered species of animals (Musser et al. 2005, Luu et al. 2013, Luu et al. 2016, Luu et al. 2017). There is an abundance of suitable mosquito habitats, making this region suitable for their development. Epidemiological and entomological studies previously have been carried out in Khammuane Province (Kobayashi et al. 1997, 2000; Toma et al. 2002), including field work in Ban Natan Region (Rueda et al. 2015). There is a lack of information about species vectors, and the major work for primary vectors still has not been accomplished in the Lao PDR.

In order to build on the knowledge regarding emerging and re-emerging vector-borne diseases, an improved

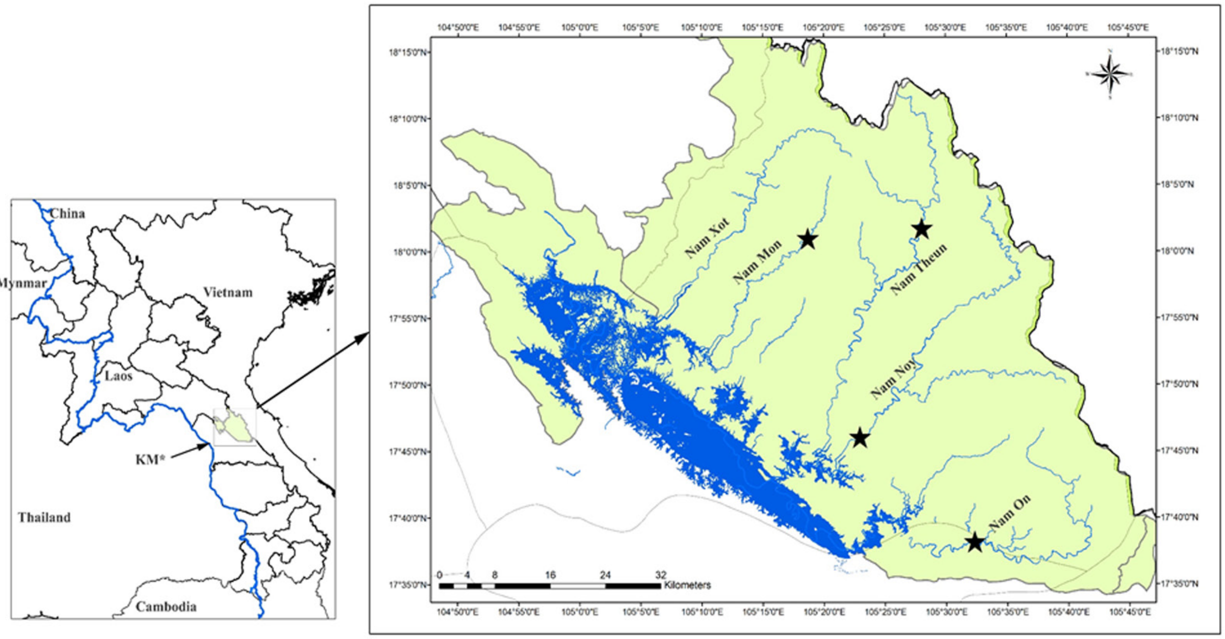

Figure 1. Map showing sampling locations of mosquitoes in Lao PDR. KM = Khammuane Province; Black stars = sampling locations; Blue line = Mekong River and its tributaries.

understanding of species composition, vector status of arthropod insects, together with increased understanding of their bionomics and ecology, we chose locations along the rivers in NNT NPA to support the mapping of health risk and vector-control interventions in the Lao PDR. We report here an extensive surveillance of mosquitoes conducted in Khammuane Province in remote areas along the rivers of Nam Theum, Nam Mon, Nam Noy, and Nam On. This study provides information about mosquito fauna of the Khammuane Province, in addition to the surveillance records of Rueda et al. (2015). Molecular identifications for some specimens of 23 mosquito species were conducted using the cytochrome c oxidase subunit I (*COI*), barcode region. Species recorded were combined with other published reports and an updated checklist of mosquitoes from Lao PDR is presented.

MATERIALS AND METHODS

Study area and specimen collection

Mosquito adult and larval collections were carried out in the Nakai Nam Theun National Protected Area (NNT NPA), known as the Watershed Management and Protection Authority (WMPA), located in Nakai District, Khammuane Province, Lao PDR. Khammuane Province, located in the south-central part of the country, is bordered by Borlikhamxay Province to the north and northwest, Vietnam to the east, Savannakhet Province to the south, and Thailand to the west. It is located about 350 km south of the capital, Vientiane. It has a tropical climate, with a temperature range of 30-34° C, and average humidity of 60-80%.

Immature and adult mosquitoes were sampled along the Nam Theun River (18.03333°N, 105.4666667°E) in January, 2012; Nam Noy River (17.768548°N, 105.381989°E) in December, 2011, March-April, 2012, February, March, May, August, November, December, 2017, and February-March, 2018; Nam Mon River (17.99416°N, 105.276664°E) in March, 2012; and Nam On River (17.636658°N, 105.538699°E) in May, 2017 (Figure 1).

Adult mosquitoes were collected using mouth aspirators, insect nets, and human-baited double net (HDN) traps (Tangena et al. 2015). Larval and pupal collections were carried out in temporary water habitats including rock pools, tree holes, and bamboo traps. They were collected using a standard larval dipper (350 ml, 13 cm diameter; BioQuip, Rancho Dominguez, CA, U.S.A.) and carefully transferred into a WhirlPak® plastic bag (BioQuip, CA, U.S.A.) using pipettes to transport back to the laboratory. Some specimens were individually link-reared to adults, as morphological voucher specimens for this work.

Morphological identification

Emerged adults were pinned on paper points, each given a unique collection number, properly labeled, and identified using diagnostic morphological characters (Rattanarithikul et al. 2005, 2006a, 2006b, 2007, 2010). All samples were separated by species, sex, and site. Whole mosquitoes or their legs were stored for molecular identifications. Voucher specimens were deposited at the Entomology Collection, Institut Pasteur du Laos (IPL), Vientiane, Lao PDR and at the SI-NMNH. The surveillance data records will be incorporated into the MosquitoMap section of VectorMap (http://vectormap.si.edu/).

Molecular identification

DNA from single legs plucked from pinned adults or whole ethanol preserved adults was extracted using the Macherey-Nagel NucleoSpin® Tissue (GmbH & Co. KG, Germany) according to manufacturer's instructions. The fragment of mtDNA *COI* gene was amplified using the PCR Master Mix 2X (Promega Corporation, U.S.A.) utilizing LCO1490 and HCO2198 primers (Folmer et al. 1994). The PCR protocol consisted of a 1 min denaturation at 94° C and five cycles at 94° C for 40 s, 45° C for 40 s, and 72° C for 1 min, followed by 30 cycles at 94° C for 40 s, 49° C for 40 s, and 72° C for 1 min and 5-min extension at 72° C. PCR amplicons were electrophoresed in 1.5% TAE agarose gels stained with GelRed Nucleic Acid Gel Stain (Biotium Inc., Hayward, U.S.A.), and then the PCR products were cleaned by adding ExosapIT® (USB Co, Cleveland, OH, U.S.A.). Samples were put back into the thermocycler and run at 37° C for 30 min, followed by 80° C for 15 min.

All sequencing reactions were carried out in both directions using the original primers and the Big Dye Terminator Kit v.3.1 (PE Applied Biosystems, Warrington, England, UK), analyzed on an ABI Prism 3500xL - Avant Genetic Analyzer (Applied Biosystems, Foster City, CA, U.S.A.). Sequences were edited in Sequencher™ v.5.4.6 (Genes Codes Co, Ann Arbor, MI, U.S.A.), and then aligned using Geneious 9.1.6 (Kearse et al. 2012). A bootstrapped (Felsenstein 1985) Neighbor Joining tree (Saitou and Nei 1987) was employed based on 5,000 replicates. The evolutionary distances were calculated using the Kimura-2 parameters method (Kimura 1980) conducted in MEGA v.7 (Kumar et al. 2016).

RESULTS

Biodiversity survey

The mosquito larval and pupal habitats along the Nam Mon, Nam Theun, Nam On, and Nam Noy rivers included bamboo nodes (with stored rainwater), temporary pools, and water pockets along the edges of rivers. A total of 6,736 specimens of mosquitoes were collected (Table 1), of which 5,318 (79%) were identified to the species level. The identities of 9.9% (667 specimens) could only be determined to the genus or subgenus level and 11.1% (751 specimens) consist of a group or a complex of species.

The collected samples belonged to 54 mosquito taxa in 11 genera: *Aedes* Meigen, *Anopheles* Meigen, *Armigeres* Theobald, *Culex* Linnaeus, *Heizmannia* Ludlow, *Lutzia* Theobald, *Topomyia* Leicester, *Toxorhynchites* Theobald, *Tripteroides* Giles, *Udaya* Thurman, and *Uranotaenia* Lynch Arribalzaga (Table 1). *Aedes macfarlanei* (Edwards) (n = 1,845) and *Ae. elsiae* (Barraud) (n = 1,803) were the most dominant species, followed by *Ae. albopictus* (Skuse) (n = 1,123). DNA barcodes were generated for 34 specimens (Genbank numbers: MH427551-MH427584). Comparison of all these sequences in conjunction with morphological analysis allowed the verification of 23 species in eight genera, including *Aedes*, *Armigeres*, *Culex*, *Heizmmania*, *Lutzia*, *Udaya*, *Toxorhynchites*, and *Tripteroides*. *Aedes vittatus* (Bigot) (MG242527 and KU380451 - query cover 100%, identity 99%), *Ar. subalbatus* (Coquillett) (KJ768109 and KM502248 - query cover 100%, identity 100%), *Cx. bicornutus* (Theobald) (AB738196 and AB738259 - query cover 100%, identity 99%), *Cx. pallidothorax* Theobald (LC054474 and AB738170 - query cover 100%, identity 100%), and *Lt. vorax* Edwards (LC054507 and LC054509 - query cover 100%, identity 99%) were compared to sequences already published in the Genbank. *Culex bicornutus* and *Cx. pallidothorax* were identified initially by morphological characters as *Cx.* Mammilifer Group and *Cx.* Wilfredi Group, and barcode sequences allowed us to correctly identify these two species. *Armigeres*, *Culex*, *Heizmmania*, and *Tripteroides* require further morphological and molecular analyses to clarify the species identities (Figures 2 and 3).

Mosquito list from Lao PDR

An updated checklist of mosquitoes in Lao PDR includes a total of 170 species (Table 2). They consist of 19 genera, namely *Aedes* (42 species), *Anopheles* (42), *Armigeres* (25), *Coquillettidia* (2), *Culex* (23), *Ficalbia* (1), *Heizmmania* (5), *Hodgesia* (1), *Lutzia* (2), *Mansonia* (4), *Mimomyia* (2), *Orthopodomyia* (1), *Topomyia* (1), *Toxorhynchites* (4), *Tripteroides* (3), *Udaya* (1), *Uranotaenia* (3), and *Verralina* (3).

Aedes (*Christophersiomyia*) sp., *Ae.* (*Hopkinsius*) *albocinctus* (Barraud), *Ae.* (*Collessius*) *elsiae*, *Ae.* (*Gilesius*) sp., *Cx.* (*Culex*) Mimeticus Complex, *Cx.* (*Culiciomyia*) *pallidothorax*, *Cx.* (*Culiciomyia*) Complex, *Cx.* (*Lophoceraomyia*) *bicornutus*, *Hz.* (*Mattinglyia*) *achaetae* (Leicester), *Hz.* (*Mattinglyia*) sp., *Lt.* (*Metalutzia*) *halifaxii* (Theobald), *Tx.* (*Toxorhynchites*) *gravely* (Edwards), *Tx.* (*Toxorhynchites*) *sunthorni* Thurman, *Tp.* (*Tripteroides*) Complex, and *Ur.* (*Uranotaenia*) *macfarlanei* Edwards are new records for the Lao PDR (Tables 1 and 2).

Voucher specimens examined

The vouchers are available at the entomology collections of the SI-NMNH and IPL. Morphological identifications are listed alphabetically by genus, subgenus, and species, including their collection numbers, as shown in supplementary data 1. Some mosquito specimens had been used for virus screening studies while other whole mosquitoes were used for DNA extraction; therefore, they are not available in the collections.

DISCUSSION

Previous mosquito surveys in Lao PDR

Lao PDR have been considered endemic for malaria (Pholsena 1992), however scientific information about its prevalence is unknown and the mosquito vectors have not yet been identified accurately (Toma et al. 2002). Study of epidemiology of malaria in Khammuane Province has been supported since 1995 by the Japan International Cooperation Agency (JICA) with the cooperation of the Institute of Malariology, Parasitology, and Entomology (IMPE), Vientiane, Lao PDR (Kobayashi et al. 1997).

Through JICA support in Khammuane Province, *Anopheles* species were collected from forest villages (Kobayashi et al. 1997) and in a location surrounded by rice fields (Kobayashi et al. 2000). In the same province, Toma et al. (2002) conducted an epidemiological study and collected 28 anopheline species in an endemic village area, where *An.*

Table 1. Summary of collected mosquito adults and larvae in Nam Mon, Nam Theun, Nam On, and Nam Noy Rivers, Nakai District, Khammuane Province, Lao PDR. RP = rock pool; BT = bamboo trap; AD = adult collection using mouth aspirators, insect nets and/or human-baited double net (HDN) trap. Species in bold are new records in Lao PDR.

Species	Nam Mon	RP	BT	AD	Nam Theun	RP	BT	AD	Nam On	RP	BT	AD	Nam Noy	RP	BT	AD
Aedes (*Bruceharrisonius*) *greeni* (Theobald)	-	-	-	-	-	-	-	-	-	-	-	-	1♀	-	-	X
Aedes (_Christophersiomyia_) sp.	-	-	-	-	-	-	-	-	-	-	-	-	1♀	-	-	X
Aedes (_Collessius_) _elsiae_ (Barraud)	16♀, 13♂	X	-	-	481♀, 464♂	X	-	-	3♀, 4♂	X	-	-	436♀, 386♂	X	X	-
Aedes (*Collessius*) *macfarlanei* (Edwards)	106♀, 89♂	X	-	-	161♀, 137♂	X	-	-	292♀, 228♂	X	-	-	439♀, 393♂	X	-	X
Aedes (*Downsiomyia*) *ganapathi* Colless	-	-	-	-	-	-	-	-	-	-	-	-	3♀	-	-	X
Aedes (*Downsiomyia*) sp.	-	-	-	-	-	-	-	-	-	-	-	-	1♀	-	-	X
Aedes (*Fredwardsius*) *vittatus* (Bigot)	-	-	-	-	-	-	-	-	-	-	-	-	13♀, 9♂	X	-	-
Aedes (_Gilesius_) sp.	-	-	-	-	13♀, 14♂	X	-	-	-	-	-	-	-	-	-	-
Aedes (_Hopkinsius_) _albocinctus_ (Barraud)	-	-	-	-	-	-	-	-	-	-	-	-	1♀	-	-	X
Aedes (*Hulecoeteomyia*) *chrysolineatus* (Theobald)	2♀, 3♂	X	-	-	11♀, 10♂	X	-	-	-	-	-	-	-	-	-	-
Aedes (*Hulecoeteomyia*) *saxicola* Edwards	1♀	X	-	-	7♀, 10♂	X	-	-	-	-	-	-	12♀	-	-	X
Aedes (*Phagomyia*) *prominens* (Barraud)	-	-	-	-	-	-	-	-	-	-	-	-	4♀	X	-	X
Aedes (*Phagomyia*) sp.	-	-	-	-	-	-	-	-	-	-	-	-	2♀	-	-	X
Aedes (*Stegomyia*) *albopictus* (Skuse)	3♀	X	-	-	-	-	-	-	1♀	X	-	-	790♀, 329♂	X	X	X
Aedes (*Stegomyia*) *desmotes* Giles	-	-	-	-	-	-	-	-	-	-	-	-	34♀, 2♂	-	-	X
Aedes (*Stegomyia*) *gardnerii imitator* (Leicester)	-	-	-	-	-	-	-	-	-	-	-	-	1♀	-	-	X
Aedes (*Stegomyia*) *malayensis* (Colless)	-	-	-	-	-	-	-	-	-	-	-	-	60♀, 40♂	X	-	X
Aedes sp.	3♀, 1♂	X	-	-	-	-	-	-	-	-	-	-	71♀, 37♂	X	-	X
Anopheles (*Cellia*) Maculatus Group	1♀, 5♂	X	-	-	2♀, 6♂	X	-	-	1♀	X	-	-	2♂	X	-	-
Anopheles sp.	1♀, 2♂	X	-	-	-	-	-	-	-	-	-	-	8♀, 6♂	X	-	X
Armigeres (*Armigeres*) *subalbatus* (Coquillett)	-	-	-	-	-	-	-	-	-	-	-	-	2♀	-	-	X
Armigeres (*Armigeres*) sp.	-	-	-	-	-	-	-	-	-	-	-	-	50♀	-	-	X
Armigeres (*Leicesteria*) *longipalpis* (Leicester)	-	-	-	-	-	-	-	-	-	-	-	-	5♀, 5♂	X	X	X
Armigeres (*Leicesteria*) sp.	-	-	-	-	-	-	-	-	-	-	-	-	8♀	X	-	X
Armigeres sp.	-	-	-	-	-	-	-	-	-	-	-	-	5♀	-	-	X
Culex (_Culex_) Mimeticus Complex	1♀	X	-	-	1♂	X	-	-	-	-	-	-	-	-	-	-
Culex (*Culex*) Mimulus Complex	5♀, 3♂	X	-	-	19♀, 24♂	X	-	-	26♀, 18♂	X	-	-	198♀, 159♂	X	X	-
Culex (*Culex*) *vishnui* Theobald	-	-	-	-	-	-	-	-	-	-	-	-	7♀	-	-	X
Culex (*Culiciomyia*) *nigropunctatus* Edwards	66♀, 32♂	X	-	-	-	-	-	-	-	-	-	-	17♀, 22♂	X	-	-
Culex (_Culiciomyia_) _pallidothorax_ Theobald	-	-	-	-	-	-	-	-	-	-	-	-	5♀	-	X	-
Culex (_Culiciomyia_) Complex	-	-	-	-	-	-	-	-	-	-	-	-	143♀, 115♂	X	X	X
Culex (_Lophoceraomyia_) _bicornutus_ (Theobald)	-	-	-	-	-	-	-	-	1♀	X	-	-	-	-	-	-
Culex (*Lophoceraomyia*) Mammilifer and Wilfredi Groups	11♀, 5♂	X	-	-	2♂	X	-	-	10♀, 2♂	X	-	-	35♀, 10♂	-	-	X
Culex (*Oculeomyia*) *sinensis* Theobald	-	-	-	-	1♂	X	-	-	-	-	-	-	-	-	-	-
Culex sp.	26♀, 9♂	X	-	-	8♀, 5♂	X	-	-	2♀, 6♂	X	-	-	34♀, 57♂	X	X	X
Heizmannia (*Heizmannia*) sp.	-	-	-	-	-	-	-	-	-	-	-	-	157♀	X	-	X
Heizmannia (_Mattinglyia_) _achaetae_ (Leicester)	-	-	-	-	-	-	-	-	-	-	-	-	55♀	-	-	X
Heizmannia (_Mattinglyia_) sp.	-	-	-	-	-	-	-	-	-	-	-	-	31♀	-	-	X
Heizmannia sp.	-	-	-	-	-	-	-	-	-	-	-	-	9♀	X	-	X
Lutzia (_Metalutzia_) _halifaxii_ (Theobald)	-	-	-	-	-	-	-	-	1♀	X	-	-	-	-	-	-
Lutzia (*Metalutzia*) *vorax* Edwards	7♀, 14♂	X	-	-	2♀	X	-	-	1♀	X	-	-	33♀, 31♂	X	X	-
Lutzia sp.	-	-	-	-	-	-	-	-	-	-	-	-	1♀	-	-	X
Topomyia sp.	-	-	-	-	-	-	-	-	-	-	-	-	1♀	-	-	X
Toxorhynchites (_Toxorhynchites_) _gravelyi_ (Edwards)	-	-	-	-	-	-	-	-	-	-	-	-	1♀, 1♂	-	X	-
Toxorhynchites (_Toxorhynchites_) _sunthorni_ Thurman	-	-	-	-	-	-	-	-	-	-	-	-	5♂	X	X	-
Toxorhynchites sp.	-	-	-	-	-	-	-	-	-	-	-	-	2♀	-	X	X
Tripteroides (*Rachionotomyia*) sp.	-	-	-	-	-	-	-	-	-	-	-	-	1♂	-	X	-
Tripteroides (_Tripteroides_) Complex	-	-	-	-	-	-	-	-	-	-	-	-	22♀	-	X	X
Tripteroides sp.	-	-	-	-	-	-	-	-	-	-	-	-	1♀	-	-	X
Udaya argyrurus (Edwards)	-	-	-	-	-	-	-	-	-	-	-	-	1♀	-	-	X
Uranotaenia (*Pseudoficalbia*) *novobscura* Barraud	-	-	-	-	-	-	-	-	-	-	-	-	4♂	X	-	-
Uranotaenia (*Pseudoficalbia*) sp.	-	-	-	-	-	-	-	-	-	-	-	-	1♀	-	-	X
Uranotaenia (_Uranotaenia_) _macfarlanei_ Edwards	-	-	-	-	-	-	-	-	-	-	-	-	3♀, 1♂	X	-	-
Uranotaenia (*Uranotaenia*) sp.	-	-	-	-	-	-	-	-	-	-	-	-	1♀	-	-	X

dirus and *An. minimus* were identified as vectors by detecting sporozoite in mosquito salivary glands. Subsequently, a preliminary malaria survey was conducted in Bolikhamxay, Champasak, Luang Prabang, Saravan, Savannakhet, Sayaboury, Sekong, and Vientiane Provinces (Vythilingam et al. 2001). Vythilingam et al. (2003) carried out a longitudinal study on the prevalence of *Anopheles* in three villages in Sekong Province. From 2002 to 2004, studies were conducted to determine the malaria vectors in Attapeu Province (Sidavong et al. 2004, Vythilingam et al. 2005), and a list of all mosquito species found in that province was reported by Vythilingam et al. (2006). We collected only few specimens (34) of *Anopheles* species. Half were identified as *An.* (*Cel.*) Maculatus Group, and the remainder were not possible to identify to species, named as *An.* sp. (Table 1).

In a diversity mosquito survey, Rueda et al. (2015)

Table 2. Updated checklist of mosquito species from Lao PDR. # indicates observed in field collection.

	Species	References
1	*Aedes* (*Aedimorphus*) *alboscutellatus* Theobald	Vythilingam et al. 2006; WRBU 2018; Rueda et al. 2015
2	*Aedes* (*Aedimorphus*) *caecus* Theobald	Tangena et al. 2017
3	*Aedes* (*Aedimorphus*) *orbitae* Edwards	Tangena et al. 2017
4	*Aedes* (*Aedimorphus*) *pipersalatus* (Giles)	Vythilingam et al. 2006; WRBU 2018
5	*Aedes* (*Aedimorphus*) *vexans* (Meigen)	Vythilingam et al. 2006; WRBU 2018; Rueda et al. 2015
6	*Aedes* (*Borichinda*) *cavernicola* Rattanarithikul and Harbach	Tangena et al. 2017
7	*Aedes* (*Bothaella*) *eldridgei* Reinert	Rueda et al. 2015
8	*Aedes* (*Bothaella*) *helenae* Reinert	Tangena et al. 2017
9	*Aedes* (*Bruceharrisonius*) *greenii* (Theobald)	Tangena et al. 2017, #
10	*Aedes* (*Collessius*) *elsiae* (Barraud)	**new record**
11	*Aedes* (*Collessius*) *macfarlanei* (Edwards)	WRBU 2018, #
12	*Aedes* (*Danielsia*) *albotaeniata* (Leicester)	Tangena et al. 2017
13	*Aedes* (*Diceromyia*) *iyengari* Edwards	Vythilingam et al. 2006; WRBU 2018
14	*Aedes* (*Downsiomyia*) *ganapathi* Colless	Rueda et al. 2015, #
15	*Aedes* (*Downsiomyia*) *harinasutai* Knight	Rueda et al. 2015
16	*Aedes* (*Downsiomyia*) *inermis* Colless	Tangena et al. 2017
17	*Aedes* (*Downsiomyia*) *niveus* (Ludlow)	WRBU 2018
18	*Aedes* (*Fredwardsius*) *vittatus* (Bigot)	Tsuda et al. 2002; WRBU 2018; Tangena et al. 2017; #
19	*Aedes* (*Hopkinsius*) *albocinctus* (Barraud)	**new record**
20	*Aedes* (*Hulecoeteomyia*) *chrysollineatus* (Theobald)	Vythilingam et al. 2006; WRBU 2018; Rueda et al. 2015; Tangena et al. 2017; #
21	*Aedes* (*Hulecoeteomyia*) *formosensis* Yamada	Rueda et al. 2015
22	*Aedes* (*Hulecoeteomyia*) *reinerti* Rattanarithikul and Harrison	Rueda et al. 2015; Tangena et al. 2017
23	*Aedes* (*Hulecoeteomyia*) *saxicola* Edwards	Tangena et al. 2017; #
24	*Aedes* (*Kenknightia*) *dissimilis* (Leicester)	Rueda et al. 2015; Tangena et al. 2017
25	*Aedes* (*Kenknightia*) *harbachi* Reinert	Tangena et al. 2017
26	*Aedes* (*Lorrainea*) *fumidus* Edwards	Tangena et al. 2017
27	*Aedes* (*Mucidus*) *quasiferinus* Mattingly	Tangena et al. 2017
28	*Aedes* (*Neomelaniconion*) *lineatopennis* (Ludlow)	Rattanarithikul et al. 2010
29	*Aedes* (*Paraedes*) *ostentatio* (Leicester)	Rattanarithikul et al. 2010
30	*Aedes* (*Phagomyia*) *khazani* Edwards	Apiwathnasorn 1986; Tangena et al. 2017
31	*Aedes* (*Phagomyia*) *prominens* (Barraud)	Apiwathnasorn 1986; Rueda et al. 2015; Tangena et al. 2017; #
32	*Aedes* (*Stegomyia*) *aegypti* (Linnaeus)	Apiwathnasorn 1986; Tsuda et al. 2002; Hiscox et al. 2013a,b; Rueda et al. 2015; WRBU 2018; Tangena et al. 2017
33	*Aedes* (*Stegomyia*) *albopictus* (Skuse)	Apiwathnasorn 1986; Vythilingam et al. 2006; Chen-Hussey 2012; Hiscox et al. 2013a,b; Rueda et al. 2015; Tangena et al. 2017; Motoki et al. 2018; #
34	*Aedes* (*Stegomyia*) *annandalei* Theobald	Tangena et al. 2017
35	*Aedes* (*Stegomyia*) *craggi* (Barraud)	Tangena et al. 2017
36	*Aedes* (*Stegomyia*) *desmotes* Giles	Tangena et al. 2017; #
37	*Aedes* (*Stegomyia*) *gardnerii imitator* (Leicester)	Vythilingam et al. 2006; Tangena et al. 2017; #
38	*Aedes* (*Stegomyia*) *malayensis* Colless	Motoki et al.2018; #
39	*Aedes* (*Stegomyia*) *pseudalbopictus* (Borel)	Vythilingam et al. 2006; WRBU 2018; #
40	*Aedes* (*Stegomyia*) *pseudoscutellaris* Theobald	Rueda et al. 2015
41	*Aedes* (*Stegomyia*) *seatoi* Huang	Tangena et al. 2017
42	*Aedes* (*Tewarius*) *pseudonummatus* Reinert	Rueda et al. 2015
43	*Anopheles* (*Anopheles*) *albotaeniatus* (Theobald)	Vythilingam et al. 2001
44	*Anopheles* (*Anopheles*) *argyropus* (Swellengrebel)	Hii and Rueda 2013
45	*Anopheles* (*Anopheles*) *baezai* Gater	Chen-Hussey 2012; Tangena et al. 2017
46	*Anopheles* (*Anopheles*) *baileyi* Edwards	Apiwathnasorn 1986; WRBU 2018; Tangena et al. 2017
47	*Anopheles* (*Anopheles*) *barbirostris* Van der Wulp	Hii and Rueda 2013; Lefebvre 1938; Vythilingam et al. 2001, 2006; Sidavong et al. 2004; WRBU 2018
48	*Anopheles* (*Anopheles*) *barbumbrosus* Strickland and Chowdhury	Kobayashi et al. 1997; Toma et al. 2002; Tangena et al. 2017
49	*Anopheles* (*Anopheles*) *donaldi* Reid	Hii and Rueda 2013; Vythilingam et al. 2006; Sidavong et al. 2004; WRBU 2018
50	*Anopheles* (*Anopheles*) *hodgkini* Reid	Toma et al. 2002; Chen-Hussey 2012; Suwonkerd et al. 2013; Tangena et al. 2017

Table 2 continued...

collected 43 taxa in Ban Natan area, Khammuane Province and reported a total of 101 species from Laos, including ten newly recorded *Aedes* and *Orthopodomyia* species. In our survey, we identified 14% of the same taxa collected by Rueda et al (2015). Their survey was employed in the west of Khammuane Province, in the cave environment close to the villages, and our surveys were done in the eastern region along the Nam Theun, Nam Mon, Nam Noy, and Nam On rivers.

Recently, Tangena et al. (2017) reported 51 additional new records to the list of Lao PDR mosquito fauna from Luang Prabang Province, including *Aedes*, *Anopheles*, *Armigeres*, *Culex*, *Heizmania*, *Lutzia,* and *Udaya* species (42 species), five species complexes and four specimens identified

Table 2. Continued.

50	*Anopheles* (*Anopheles*) *hodgkini* Reid	Toma et al. 2002; Chen-Hussey 2012; Suwonkerd et al. 2013; Tangena et al. 2017
51	*Anopheles* (*Anopheles*) *hyrcanus*(Pallas)	Sidavong et al. 2004
52	*Anopheles* (*Anopheles*) *insulaeflorum* (Swellengrebel and Swellengrebel de Graaf)	Tangena et al. 2017
53	*Anopheles* (*Anopheles*) *separatus* (Leicester)	Tangena et al. 2017
54	*Anopheles* (*Anopheles*) *sinensis* Wiedemann	Hewitt et al. 2013; Lefebvre 1938
55	*Anopheles* (*Anopheles*) *umbrosus* (Theobald)	Vythilingam et al. 2003; Marcombe e tal. 2017; Tangena et al. 2017
56	*Anopheles* (*Anopheles*) *whartoni* Reid	Tangena et al. 2017
57	*Anopheles* (*Cellia*) *aconitus* Doenitz	Gaschen 1934; Hii and Rueda 2013; Apiwathnasorn 1986; Vythilingam et al. 2001; Sidavong et al. 2004; Toma et al. 2002; Vythilingam et al. 2003; Vythilingam et al. 2006; Hiscox et al. 2013a; WRBU 2018; Marcombe et al. 2017; Tangena et al. 2017
58	*Anopheles* (*Cellia*) *annularis* Van der Wulp	Hii and Rueda 2013
59	*Anopheles* (*Cellia*) *culicifacies* Giles	Hii and Rueda 2013; Gaschen 1934; WRBU 2018; Kobayashi et al. 1997; Toma et al. 2002; Chen-Hussey 2012; Tangena et al. 2017
60	*Anopheles* (*Cellia*) *dirus* Peyton and Harrison	Hii and Rueda 2013; Tsuda et al. 2002; Vythilingam et al. 2001, 2003, 2005, 2006; WRBU 2018; Kobayashi et al. 1997, 2000; Toma et al. 2002; Sidavong et al. 2004; Chen-Hussey 2012; Suwonkerd et al. 2013; Tangena et al. 2017
61	*Anopheles* (*Cellia*) *dravidicus* Christophers	Hii and Rueda 2013; Vythilingam et al. 2006; Sidavong et al. 2004; WRBU 2018; Marcombe et al. 2017
62	*Anopheles* (*Cellia*) *epiroticus* Linton and Harbach	Tangena et al. 2017
63	*Anopheles* (*Cellia*) *harrisoni* Harbarch and Manguin	WRBU 2018
64	*Anopheles* (*Cellia*) *indefinitus* (Ludlow)	Hii and Rueda 2013; WRBU 2018
65	*Anopheles* (*Cellia*) *jamesii* Theobald	Apiwathnasorn 1986; Kobayashi et al. 2000; Vythilingam et al. 2001; Toma et al. 2002; Chen-Hussey 2012; Hii and Rueda 2013; WRBU 2018; Tangena et al. 2017
66	*Anopheles* (*Cellia*) *jeyporiensis* James	Hii and Rueda 2013; Gaschen 1934; Lefebvre 1938; Vythilingam et al. 2003; WRBU 2018; Apiwathnasorn 1986; Kobayashi et al. 1997; Chen-Hussey 2012; Suwonkerd et al. 2013; Tangena et al. 2017
67	*Anopheles* (*Cellia*) *karwari* (James)	Hii and Rueda 2013; Vythilingam et al. 2003, 2005; Sidavong et al. 2004; WRBU 2018
68	*Anopheles* (*Cellia*) *kochi* Donitz	Hii and Rueda 2013; Lefebvre 1938; Apiwathnasorn 1986; Kobayashi et al. 1997, 2000; Vythilingam et al. 2001, 2003, 2006; Sidavong et al. 2004; WRBU 2018; Toma et al. 2002; Chen-Hussey; Marcombe et al. 2017; Tangena et al. 2017
69	*Anopheles* (*Cellia*) *maculatus* Theobald	Gaschen 1934; Lefebvre 1938; Apiwathnasorn 1986; Kobayashi et al. 1997; Vythilingam et al. 2001, 2003, 2005, 2006; Sidavong et al. 2004; Toma et al. 2002; Chen-Hussey 2012; Hii and Rueda 2013; Suwonkerd et al. 2013; WRBU 2018; Marcombe et al. 2017
70	*Anopheles* (*Cellia*) *minimus* Theobald	Lefebvre 1938; Apiwathnasorn 1986; Kobayashi et al. 1997, 2000; Vythilingam et al. 2001, 2003, 2005, 2006; Toma et al. 2002; Sidavong et al. 2004; Chen-Hussey 2012; Hii and Rueda 2013; Suwonkerd et al. 2013; WRBU 2018; Marcombe et al. 2017
71	*Anopheles* (*Cellia*) *nivipes* (Theobald)	Vythilingam et al. 2001, 2003, 2006; Sidavong et al. 2004; WRBU 2018; Marcombe et al. 2017
72	*Anopheles* (*Cellia*) *notanandai* Rattanarithikul and Green	Hii and Rueda 2013; Vythilingam et al. 2006; Sidavong et al. 2004; WRBU 2018
73	*Anopheles* (*Cellia*) *pallidus* Theobald	Vythilingam et al. 2001, 2003; Sidavong et al. 2004
74	*Anopheles* (*Cellia*) *pampanai* Buttiker and Beales	Vythilingam et al. 2001, 2006; Toma et al. 2002; Sidavong et al. 2004; Chen-Hussey 2012; WRBU 2018; Marcombe et al. 2017; Tangena et al. 2017
75	*Anopheles* (*Cellia*) *philippinensis* Ludlow	Lefebvre 1938; Apiwathnasorn 1986; Kobayashi et al. 1997, 2000; Vythilingam et al. 2001, 2003, 2006; Sidavong et al. 2004; Toma et al. 2002; Chen-Hussey 2012; Hii and Rueda 2013; Hiscox et al. 2013a; Suwonkerd et al. 2013; WRBU 2018; Marcombe et al. 2017; Tangena et al. 2017
76	*Anopheles* (*Cellia*) *pseudowillmori* (Theobald)	Hii and Rueda 2013; Vythilingam et al. 2006; Sidavong et al. 2004; WRBU 2018; Rueda et al. 2015
77	*Anopheles* (*Cellia*) *rampae* Harbach and Somboon	Marcombe et al. 2017
78	*Anopheles* (*Cellia*) *sawadwongporni* Rattanarithikul and Green	Vythilingam et al. 2006; Sidavong et al. 2004; WRBU 2018; Marcombe et al. 2017
79	*Anopheles* (*Cellia*) *splendidus* Koidzumi	Vythilingam et al. 2001, 2003, 2006; Sidavong et al. 2004; WRBU 2018
80	*Anopheles* (*Cellia*) *subpictus* Grassi	Hii and Rueda 2013; Vythilingam et al. 2001
81	*Anopheles* (*Cellia*) *sundalcus* (Rodenwaldt)	Hii and Rueda 2013
82	*Anopheles* (*Cellia*) *tessellatus* Theobald	Apiwathnasorn 1986; Kobayashi et al. 1997; Vythilingam et al. 2001, 2006; Toma et al. 2002; Sidavong et al. 2004; Chen-Hussey 2012; Hii and Rueda 2013; Hiscox et al. 2013a; WRBU 2018; Marcombe et al. 2017; Tangena et al. 2017
83	*Anopheles* (*Cellia*) *vagus* Donitz	Hii and Rueda 2013; Gaschen 1934; Lefebvre 1938; Vythilingam et al. 2001, 2003, 2006; Marcombe et al. 2017
84	*Anopheles* (*Cellia*) *varuna* Iyengar	Vythilingam et al. 2001, 2003, 2006; Toma et al. 2002; Sidavong et al. 2004; WRBU 2018; Hii and Rueda 2013; Tangena et al. 2017
85	*Armigeres* (*Armigeres*) *aureolineatus* (Leicester)	WRBU 2018
86	*Armigeres* (*Armigeres*) *confusus* Edwards	Tangena et al. 2017
87	*Armigeres* (*Armigeres*) *durhami* (Edwards)	WRBU 2018
88	*Armigeres* (*Armigeres*) *foliatus* Brug	Tangena et al. 2017
89	*Armigeres* (*Armigeres*) *jugraensis* (Leicester)	Tangena et al. 2017
90	*Armigeres* (*Armigeres*) *kesseli* Ramalingam	Tangena et al. 2017

Table 2. Continued.

90	*Armigeres* (*Armigeres*) *kesseli* Ramalingam	Tangena et al. 2017
91	*Armigeres* (*Armigeres*) *kuchingensis* Edwards	Apiwathnasorn 1986; Vythilingam et al. 2006; WRBU 2018; Tangena et al. 2017
92	*Armigeres* (*Armigeres*) *laoensis* Toma and Miyagi	Toma and Miyagi 2003; WRBU 2018
93	*Armigeres* (*Armigeres*) *malayi* (Theobald)	Tangena et al. 2017
94	*Armigeres* (*Armigeres*) *moultoni* Edwards	Apiwathnasorn 1986; WRBU 2018; Tangena et al. 2017
95	*Armigeres* (*Armigeres*) *setifer* Delfinado	Vythilingam et al. 2006; WRBU 2018
96	*Armigeres* (*Armigeres*) *subalbatus* (Coquillett)	Apiwathnasorn 1986; Vythilingam et al. 2006; Hiscox 2011; Hiscox et al. 2016; WRBU 2018; Tangena et al. 2017; #
97	*Armigeres* (*Armigeres*) *theobaldi* Barraud	Vythilingam et al. 2006; WRBU 2018; Tangena et al. 2017
98	*Armigeres* (*Leicesteria*) *annulipalpis* (Theobald)	Tangena et al. 2017
99	*Armigeres* (*Leicesteria*) *annulitarsis* (Leicester)	Apiwathnasorn 1986; WRBU 2018; Tangena et al. 2017
100	*Armigeres* (*Leicesteria*) *balteatus* Macdonald	Tangena et al. 2017
101	*Armigeres* (*Leicesteria*) *digitatus* (Edwards)	Tangena et al. 2017
102	*Armigeres* (*Leicesteria*) *dolichocephalus* (Leicester)	Apiwathnasorn 1986; WRBU 2018; Tangena et al. 2017
103	*Armigeres* (*Leicesteria*) *flavus* (Leicester)	Apiwathnasorn 1986; Rueda et al. 2015; WRBU 2018; Tangena et al. 2017
104	*Armigers* (*Leicesteria*) *inchoatus* Barraud	Tangena et al. 2017
105	*Armigeres* (*Leicesteria*) *longipalpis* (Leicester)	Apiwathnasorn 1986; Rueda et al. 2015; WRBU 2018; Tangena et al. 2017; #
106	*Armigeres* (*Leicesteria*) *magnus* (Theobald)	Apiwathnasorn 1986; Rueda et al. 2015; WRBU 2018; Tangena et al. 2017
107	*Armigeres* (*Leicesteria*) *omissus* (Edwards)	Tangena et al. 2017
108	*Armigeres* (*Leicesteria*) *pectinatus* (Edwards)	WRBU 2018
109	*Armigeres* (*Leicesteria*) *traubi* Macdonald	Tangena et al. 2017
110	*Coquillettidia* (*Coquillettidia*) *crassipes* (Van der Wulp)	Vythilingam et al. 2006; WRBU 2018
111	*Coquillettidia* (*Coquillettidia*) *ochracea* (Theobald)	WRBU 2018; Rueda et al. 2015
112	*Culex* (*Culex*) *alis* Theobald	Chen-Hussey 2012; Tangena et al. 2017
113	*Culex* (*Culex*) *edwardsi* Barraudi	Tangena et al. 2017
114	*Culex* (*Culex*) *fuscocephala* Theobald	Apiwathnasorn 1986; Vythilingam et al. 2006; Chen-Hussey 2012; Hiscox et al. 2013a; WRBU 2018; Tangena et al. 2017
115	*Culex* (*Culex*) *gelidus* Theobald	Apiwathnasorn 1986; Toma et al. 2002; Vythilingam et al. 2006; Hiscox 2011; Chen-Hussey 2012; WRBU 2018; Tangena et al. 2017
116	*Culex* (*Culex*) *hutchinsoni* Barraud	Vythilingam et al. 2006; Chen-Hussey 2012; WRBU 2018; Tangena et al. 2017
117	*Culex* (*Culex*) Mimeticus Complex	**new record**
118	*Culex* (*Culex*) Mimulus Complex	Tangena et al. 2017; #
119	*Culex* (*Culex*) *perplexus* Leicester	Chen-Hussey 2012; Tangena et al. 2017
120	*Culex* (*Culex*) *pseudovishnui* Colless	Vythilingam et al. 2006; WRBU 2018
121	*Culex* (*Culex*) *quinquefasciatus* Say	Apiwathnasorn 1986; Vythilingam et al. 2006; Chen-Hussey 2012; Hiscox et al. 2013a; Rueda et al. 2015; WRBU 2018; Tangena et al. 2017
122	*Culex* (*Culex*) *sitiens* Wiedemann	Apiwathnasorn 1986; Chen-Hussey 2012; Tangena et al. 2017
123	*Culex* (*Culex*) *tritaeniorhynchus* Giles	Vythilingam et al. 2006; WRBU 2018; Rueda et al. 2015
124	*Culex* (*Culex*) *vishnui* Theobald	Vythilingam et al. 2006; WRBU 2018; Toma et al. 2002; Chen-Hussey 2012; Hiscox et al. 2013a; Tangena et al. 2017; #
125	*Culex* (*Culex*) *whitei* Barraud	Chen-Hussey 2012; Tangena et al. 2017
126	*Culex* (*Culex*) *whitmorei* (Giles)	Apiwathnasorn 1986; Toma et al. 2002; Vythilingam et al. 2006; Hiscox 2013a; Chen-Hussey 2012; WRBU 2018; Tangena et al. 2017
127	*Culex* (*Culiciomyia*) Complex	**new record**
128	*Culex* (*Culiciomyia*) *dispectus* Bram	Tangena et al. 2017;
129	*Culex* (*Culiciomyia*) *nigropunctatus* Edwards	Vythilingam et al. 2006; Chen-Hussey 2012; Rueda et al. 2015; WRBU 2018; Tangena et al. 2017; #
130	*Culex* (*Culiciomyia*) *pallidothorax* Theobald	**new record**
131	*Culex* (*Culiciomyia*) *papuensis* (Taylor)	Tangena et al. 2017
132	*Culex* (*Culiciomyia*) *termi* Thurman	Tangena et al. 2017
133	*Culex* (*Eumelanomyia*) *foliatus* Brug	Chen-Hussey 2012; Tangena et al. 2017
134	*Culex* (*Lophoceraomyia*) *binocornutus* (Theobald)	**new record**
135	*Culex* (*Lophoceraomyia*) Mammilifer and Wilfredi Groups	Tangena et al. 2017

Table 2. Continued.

136	*Culex* (*Oculeomyia*) *bitaeniorhynchus* Giles	Apiwathnasorn 1986; Vythilingam et al. 2006; Chen-Hussey 2012; Hiscox et al. 2013a; WRBU 2018; Tangena et al. 2017
137	*Culex* (*Oculeomyia*) *longicornis* Sirivanakarn	Tangena et al. 2017
138	*Culex* (*Oculeomyia*) *pseudosinensis* Colless	Vythilingam et al. 2006; WRBU 2018; Tangena et al. 2017
139	*Culex* (*Oculeomyia*) *sinensis* Theobald	Vythilingam et al. 2006; WRBU 2018; #
140	*Ficalbia minima* (Theobald)	WRBU 2018
141	*Heizmannia* (*Heizmannia*) *chengi* Lien	Tangena et al. 2017
142	*Heizmannia* (*Heizmannia*) *complex* (Theobald)	Apitwathnasorn 1986; WRBU 2018; Tangena et al. 2017
143	*Heizmannia* (*Heizmannia*) *demeilloni* Mattingly	Tangena et al. 2017
144	*Heizmannia* (*Heizmannia*) *mattinglyi* Thurman	Tangena et al. 2017
145	*Heizmannia* (*Mattinglyia*) *achaetae* (Leicester)	**new record**
146	*Hodgesia malay* Leicester	WRBU 2018
147	*Lutzia (Metalutzia) halifaxii* (Theobald)	**new record**
148	*Lutzia* (*Metalutzia*) *vorax* Edwards	Tangena et al. 2017; #
149	*Mansonia* (*Mansonioides*) *annulifera* (Theobald)	Vythilingam et al. 2006; WRBU 2018
150	*Mansonia* (*Mansonioides*) *dives* (Schiner)	Vythilingam et al. 2006
151	*Mansonia* (*Mansonioides*) *indiana* Edwards	Vythilingam et al. 2006; WRBU 2018
152	*Mansonia* (*Mansonioides*) *uniformes* (Theobald)	Vythilingam et al. 2006; WRBU 2018; Rueda et al. 2015
153	*Mimomyia* (*Mimomyia*) *chamberlaini* Ludlow	WRBU 2018
154	*Mimomyia* (*Mimomyia*) *hybidra* (Leicester)	WRBU 2018
155	*Orthopodommyia albipes* Leicester	Rueda et al. 2015
156	*Topomyia* (*Topomyia*) *gracilis* Leicester	WRBU 2018
157	*Toxorhynchites* (*Toxorhynchites*) *albipes* (Edwards)	WRBU 2018
158	*Toxorhynchites* (*Toxorhynchites*) *gravelyi* (Edwards)	**new record**
159	*Toxorhynchites* (*Toxorhynchites*) *kempi* (Edwards)	WRBU 2018
160	*Toxorhynchites* (*Toxorhynchites*) *sunthorni* Thurman	**new record**
161	*Tripteroides* (*Rachionotomyia*) *aranoides* (Theobald)	WRBU 2018
162	*Tripteroides* (*Rachionotomyia*) *ponmeki* Miyagi and Toma	Miyagi and Toma 2001; WRBU 2018
163	*Tripteroides* (*Tripteroides*) Complex	**new record**
164	*Udaya argyrurus* (Edwards)	Tangena et al. 2017; #
165	*Uranotaenia* (*Pseudoficalbia*) *nivipleura* Leicester	WRBU 2018
166	*Uranotaenia* (*Pseudoficalbia*) *novobscura* Barraud	WRBU 2018; #
167	*Uranotaenia (Uranotaenia) macfarlanei* Edwards	**new record**
168	*Verrallina* (*Verrallina*) *dux* (Dyar and Shannon)	WRBU 2018
169	*Verrallina* (*Harbachius*) *yusafi* (Barraud)	Tangena et al. 2017
170	*Verrallina* (*Verrallina*) *lugubris* (Barraud)	Tangena et al. 2017

to genus level. All voucher specimens in the report of Tangena et al. (2017), however, were unfortunately damaged or lost, and we were not able to conduct any further morphological examinations or DNA analysis of those specimens from this study. Attempts should be made to recollect specimens reported by Tangena et al. (2017) to further confirm the true identity of the species, particularly by using molecular analysis. None of the reports mentioned above aid molecular analysis, except Marcombe et al. (2017), which identified 13 *Anopheles* species/groups by molecular methods.

Lao PDR has an extensive network of rivers, such as the Mekong river and it is tributaries, which facilitate population movement within the country and between the country and its neighbors and can also be a source of vector-disease spread. The Mekong River and its tributaries, with the presence of a tropical climate, can be a good resource for development of larvae and their spread throughout the country. For these reasons, many provinces in Lao PDR remain to be sampled, particularly those border areas, due to the presence of efficient vectors and extensive population movement between Lao PDR and its neighboring countries.

Current biodiversity survey in Khammuane Province

Aedes

Most *Aedes* species were found in sympatry, in rock pools, and a few specimens in bamboo traps (Table 1). *Aedes macfarlanei* and *Ae. elsiae* were the most abundant species collected (Table 1). The biology and vector status of these two species remain unknown. Several species of *Aedes*, especially of subgenus *Stegomyia,* are major vectors of various organisms that cause human infectious diseases such as dengue, yellow fever, chikungunya, Zika viruses, and filariasis (Huang 1979, 2004, Peters 1992).

A bootstrap tree was made by adding two specimens of *Ae. malayensis* Colless (MG921175-MG921176) and two specimens of *Ae. albopictus* (MG921177-MG921178) collected in this survey already published in Motoki et al. (2018) (Figure 2). Morphological characters were corroborated with DNA barcodes to confirm *Ae. malayensis*, *Ae. albopictus* (identified in Motoki et al. 2018), *Ae. elsiae*, *Ae. saxicola*, *Ae. ganapathi*, *Ae. vittatus,* and *Ae. prominens* (Figure 2).

The subgenus *Gilesius* comprises *Ae. alius* Lien and

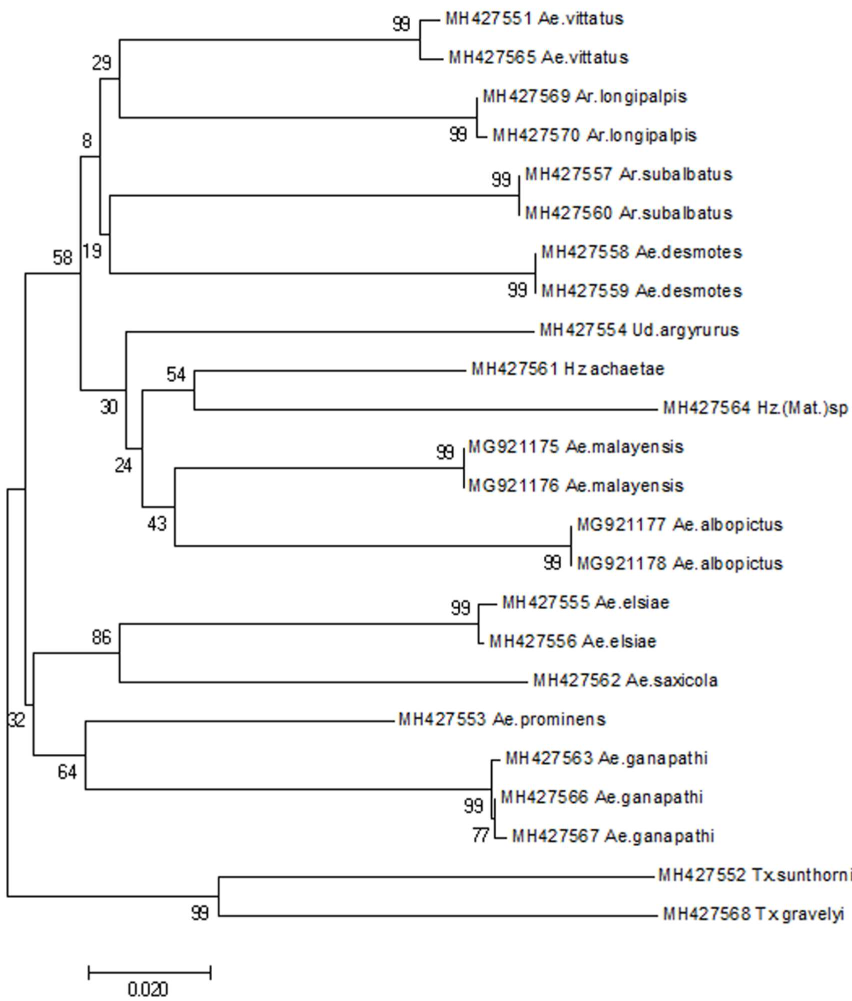

Figure 2. Bootstrapped tree of *COI* gene of *Aedes, Armigeres, Heizmannia, Udaya* and *Toxorhynchites* species (MH427551-MH427570) inferred using the neighbor-joining method based on 5,000 replicates of Tamura-Nei algorithm. Bootstrap values less than 50% are not shown. Scale bar represents sequence (%) divergence between samples. *Aedes malayensis* (MG921175-MG921176) and *Ae. albopictus* (MG921177-MG921178) were added from Motoki et al. (2018).

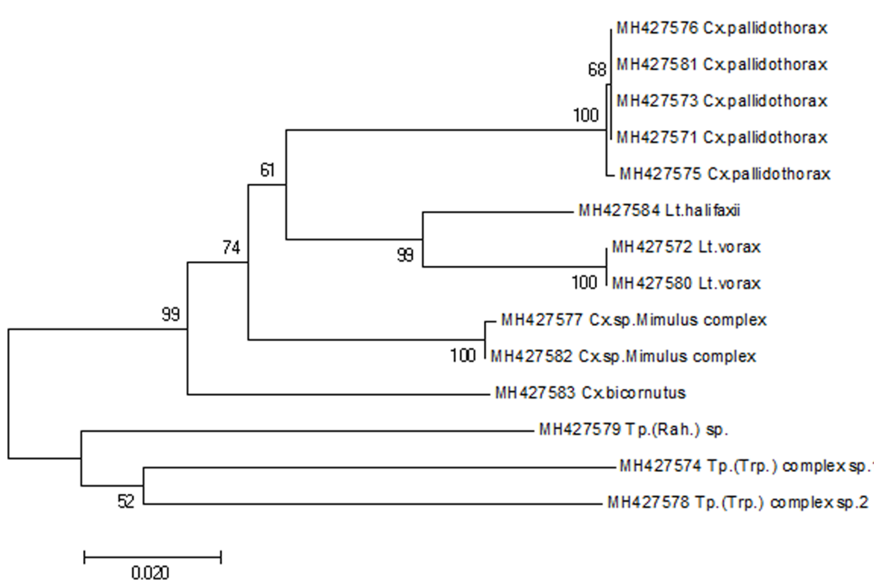

Figure 3. Bootstrapped tree of *COI* gene of *Culex*, *Lutzia* and *Tripteroides* species (MH427571-MH427584) inferred using the neighbor-joining method based on 5,000 replicates of Tamura-Nei algorithm. Bootstrap values less than 50% are not shown. Scale bar represents sequence (%) divergence between samples.

Ae. pulchriventer (Giles) (WRBU 2018). We identified 27 specimens as *Ae.* (*Gilesius*) sp. in rock pools along the Nam Theun River; however, more analyses are needed to accurately identify these specimens.

Armigeres and *Heizmannia*

All species of these two genera were found in the Nam Noy River area and most specimens were collected using manual aspirators and HDN traps, while a small number were collected from rock pools. At least four species of subgenus *Armigeres* and one species of subgenus *Leicesteria* in genus *Armigeres*, and at least five species of subgenus *Heizmannia* in genus *Heizmannia* are found in Lao PDR. *Armigeres* (*Armigeres*) *laoensis* Toma and Miyagi was described in 2003 based on samples from Khammuane Province (Toma and Miyagi 2003). Morphological characters of specimens from our collections were corroborated with DNA barcodes to confirm *Ar. subalbatus*, *Ar. longipalpis*, *Hz. Achaetae,* and *Hz.* (*Mat.*) sp (Figure 2). DNA barcodes were also generated for an additional number of samples of *Heizmannia* and *Armigeres* species. We assumed that *Hz.* (*Mat.*) sp. should be *Hz. catesi* (Lien); however, more samples should be checked molecularly and morphologically to determine if those specimens comprise only one species or a complex of species.

Armigeres subalbatus (Figure 2) has been known to be a vector of Japanese encephalitis virus (Liu et al. 2013), human filariasis in India (Das et al. 1983), and zoonotic filariasis in Malaysia (Cheong et al. 1981, Muslim et al. 2013). Furthermore, Hiscox et al. (2016) isolated a dengue 3 virus from *Ar. subalbatus* but did not conduct competency studies to confirm its status as a vector. The vector status of other species of *Armigeres* and all the species of *Heizmannia* remains unknown.

Udaya and *Topomyia*

Only one specimen was found of each genus, namely *Udaya argyrurus* (Edwards) and *Topomyia* sp. These two species were collected using manual aspirators and HDN traps (Table 1). No species of these genera appear to be of medical or economic importance to humans.

Anopheles

Numerous specimens of *Anopheles* were collected from Khammuane Province in rock pools using manual aspirators. Marcombe et al. (2017) tested insecticide resistance of *Anopheles* species in Lao PDR and identified 13 *Anopheles* species/groups by molecular methods. We collected 17 specimens of *An. maculatus* Group but could not identify the specimens to species using morphological characters. Some *Anopheles* species (*An. dirus, An. maculatus, An. minimus*) have been incriminated as vectors of malaria in Lao PDR, but the medical importance of many other anopheline species remains unknown.

Culex and *Lutzia*

Samples were collected from bamboo and rock pools using HDN traps. Various species of *Culex* are primary vectors of Japanese encephalitis virus in Thailand, Vietnam, Malaysia, and India (Gould et al. 1974, Thoa et al. 1974, Gingrich et al. 1992, Samuel et al. 1998) and vectors of filariasis in India, Sri Lanka, and Thailand (Iyengar 1938, Carter 1948, Sucharit et al. 1988, Jitpakdi et al. 1998). Larvae of *Lutzia* species are predaceous, particularly on larvae of other mosquitoes (Rattanarithikul et al. 2005). The *COI* barcode sequences from this report identified *Cx. pallidothorax* in *Cx.* (*Culiciomyia*) Complex and *Cx. bicornutus* in subgenus *Lophoceraomyia* of genus *Culex* (Figure 3). About 258 specimens of *Cx.* (*Culiciomyia*) Complex and 75 specimens of *Cx.* (*Lophoceraomyia*) spp. were identified (Table 1). Furthermore, *Lt. vorax* and *Lt. halifaxii* were identified both morphologically and molecularly (Figure 3). Additional DNA barcodes need to be generated to examine the diversity of the *Cx.* complex in Lao PDR.

Tripteroides

Little is known about the medical importance of this genus, except for a few specimens that attack and bite humans (Rattanarithikul et al. 2007). The habitats of larvae of *Tp. ponmeki* Miyagi and Toma seem to be similar to species of the genus *Armigeres* (Miyagi and Toma 2001). Larvae were found in bamboo and one adult was captured by HDN traps in our collections. *COI* barcodes identified one species of *Tp.* (*Rachionotomyia*) sp. and two species into the *Tp.* (*Tripteroides*) Complex (Figure 3).

Toxorhynchites

The species of *Toxorhynchites* from our samples were collected in rock pools and bamboo (Figures 2 and 3). *Toxorhynchites gravely* (Edwards)*, Tx. sunthorni* Thurman, and other species of the subgenus *Toxorhynchites* have been reported in Thailand. Because of their predatory behavior, usually a few larvae of other mosquitoes are found in association with the larvae of this genus in the same habitat. Larvae of *Toxorhynchites* species feed principally on the larvae of other mosquito species (Rattanarithikul et al. 2007).

Uranotaenia

Uranotaenia (*Pseudoficalbia*) *novobscura* Barraud and *Ur. (Uranotaenia) macfarlanei* Edwards were collected in rock pools or using HDN traps. One specimen of *Ur.* (*Pse.*) and one specimen of *Ur.* (*Ura.*) were not possible to identify morphologically, and they may need molecular analysis to confirm their species status. The species of genus *Uranotaenia* have not been incriminated as vectors of human pathogens; however, they have been found to be positive for certain viruses (Rattanarithikul et al. 2007).

In summary, we generated 34 sequences of *COI* barcode region of Lao PDR samples, which could aid in molecular identification of 23 species (Figures 2 and 3). A list of mosquitoes from Lao PDR was updated containing 170 species, including 15 new taxa records (Table 2). *Aedes elsiae*, one of the new records in Lao PDR, and its abundance with *Ae. macfarlanei* (Table 1) in all locations collected, require more attention in relation to their vector status. The taxonomic status of *Armigeres* and *Heizmannia* and the diversity of *Culex* species still need to be clarified. Further analysis including morphology plus molecular methods are required. We are in the process of elucidating the taxonomic status of *Armigeres* species in Lao PDR. This survey may help health personnel map out some risk areas for infectious diseases as well as vector control interventions. Nevertheless, additional studies, such as ecological surveys, are warranted on vector surveillance to improve the knowledge of the entomofauna in Lao PDR.

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