Dates	04/12 to 10/12/2023	
Mission order	IPC MO 2023/655, 2023/656, 2023/657, 2023/658, 2023/659	
Province	Stung Treng, Cambodia	
Participants	Hoem Thavry	Virology Unit, IPC
	Julia Guillebaud	
	Nuon Sithun	
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	Nov Kimsear	Entomology Unit, IPC
	Flavien Cabon	
	Pauline Van Leeuwen	University of Liege, Belgium
	Alix Nicolas	
	Julien Cappelle	CIRAD, France
	David Pleydell	
	Cherie Yu	
	Cedric Marsboom (07/12 – 10/12/2023)	Avia-GIS, Belgium
	Vincent Sluydts (07/12 – 10/12/2023)	University of Antwerpen, Belgium
	San Sovannary	Department of Wildlife and Biodiversity, Forestry Administration, MAFF
	Chhun Vanna (04/12 – 07/12/2023)	

1. Objectives

- Bats capture and sampling in 3 karst hills: Chhgnauk, Ka Ngoark and Chab Pleurng (longitudinal follow-up)
- Entomological data collection
  - Set up mosquito CDC light traps and other relevant traps
  - Collect ectoparasites on bats during sampling sessions
- Environmental sampling
  - Perform air sampling inside 2 caves for both virus detection and biodiversity assessment (3h protocol*2) and rapid air sampling using portable instrument (during bats sampling)
  - Collect bat guanos inside two selected caves (Ka Ngoark and Chab Pleurng hills)
- Implementation of protocol for bat acoustics
- Follow-up on camera traps protocol in 1 main cave: Chhgnauk

2. General comments and observations

Overall, the mission was successful and all activities were implemented as planned. The use of walkie-talkies greatly improved the coordination of activities among the different teams.

However, we have been informed on the first night by the chief of the community that we would not be allowed for two nights of trapping around Chhgnauk hill as there should be a project starting with conservation NGO (Wildlife Alliance) for bats conservation among other things. This is why we haven't been able to implement capture around the ground cave in Chhgnauk as usual on the 07/12/2023. Forestry Administration officers will discuss further so we can coordinate with all actors to pursue capture around Chhgnauk hill.

For next field mission, we will have to send also the official documents of approvals to the District authorities in addition to local authorities only as per requested by District officials visiting the site.

All comments and remarks regarding specific activities will be developed in the different sections of this report.

3. Bats capture and sampling

3.1. Bats capture effort

Only mist-nets were used during this capture session (no harp-trap). Between 2 and 4 mist-nets were used in each selected location. Capture sessions occurred twice in each hill. A total of 8 different locations were selected for bats capture (Table 1, Figure 1). However, as mentioned in section 2 of this report, "Ground cave" and "Monk cave" locations were only visited once because of restricted access; only "Corridor" location was assessed twice. Compare to previous field work, we thus trapped and sampled much fewer bats in Chhgnauk hill than previously.

Table 1: Location of trapping per site.

Hill	Location
Chhgnauk	In front of ground cave and surrounding forest
	"Monk" cave
	Corridor
Ka Ngoark	Ground cave
	Hip of the hill
	Plantation
Chab Pleurng	Forest close to pagoda
	Forest North-West from pagoda (L2)

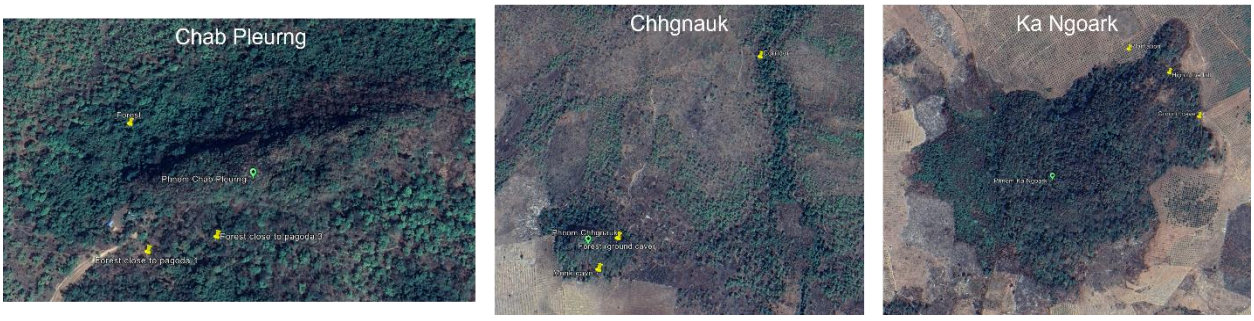

Figure 1: Trapping locations per hill.

3.2. Bats capture

- Distribution of bat capture and sampling per night and site

A total of 186 individual bats were samples over 6 nights of capture (Table 2). 8.6% (16/186), 42.5% (79/186), and 48.9% (91/186) of bats were captured in Chhgnauk, Ka Ngoark and Chab Pleurng hills respectively over 2 trapping nights. One *R. shameli* individual was recaptured on the 09/12/2023, leading Among 185 other captured bats, 90.8% (168/185) were chipped, including 110 (65%) *Rhinolophus* bats.

Table 2: Distribution of bat capture success per night and hill.

Sampling date	Chhngauk	Kar Ngaork	Chab Pleurng	Total
4-Dec	3	-	-	3
5-Dec	-	45	-	45
6-Dec	-	-	50	50
7-Dec	13	-	-	13
8-Dec	-	34	-	34
9-Dec	-	-	41	41
Total	**16 (8.6%)**	**79 (42.5%)**	**91 (48.9%)**	**186**

- Distribution of species per site

Eight genera were identified among captured and sampled bats (Table 3). *Rhinolophus* bats represented 68.3% (127/186) of total individuals and most of those identified were *R. shameli* (92.1%, 117/127). Specific barcoding (CO1 gene) will be performed for all non-identified species and to confirm some closely related species (i.e.: *R. malayanus* and *R. pusillus*).

Table 3: Distribution of identified species by site (only individuals that were sampled).

Species	Chhngauk	Kar Ngaork	Chab Pleurng	Total
Rhinolophus shameli	9	28	80	117
Taphozous melanopogon	2	34		36
Cynopterus brachyotis		2	4	6
Rhinolopus malayanus		4	1	5
Megaderma spasma	1		3	4
Rhinolophus pusillus		3	1	4
Cynopterus sphinx		3		3
Taphozous sp.		2		2
Cynopterus sp.	2			2
Eonycteris speleae			1	1
Hipposideros armiger		1		1
Lyroderma lyra		1		1
Eonycteris sp.	1			1
Rousettus sp.	1			1
Rhinolophus stheno (TBC)		1		1
Hipposideros sp.			1	1
Total	**16**	**79**	**91**	**186**

- Distribution of species per sex and age status

One individual had missing information regarding sex and age status. The table 4 presents thus the frequency of male and female and their age status for N=185 bats. Female represented 64.9% (120/185) of captured individuals. Adults represented 85.2% (201/236). Juvenile represented 15.9% (18/113) and 12.2% (15/123) of female and male individuals, respectively.

Table 4: Distribution of species per sex and status.

	Female					Male			
Species	Adult	Juvenile	Nulliparous	Parous	**Total**	Adult	Immature	Juvenile	**Total**
C. sphinx			2		2			1	1
E. speleae								1	1
H. armiger								1	1
L. lyra						1			1
M. spasma				2	2	2			2
R. pusillus						4			4
R. shameli	2	5	45	37	89	24	2	2	28
T. melanopogon			14	8	22	13		1	14
Taphozous sp.				1	1	1			1
C. brachyotis			2		2	2		2	4
R. malayanus			1	1	2	2			2
Cynopterus sp.								2	2
Eonycteris sp.								1	1
Rousettus sp.								1	1
Rhinolophus stheno?						1			1
Hipposideros sp.						1			1
Total	**2**	**5**	**64**	**49**	**120**	**51**	**2**	**12**	**65**

** One bat species was status identification missing

3.3. Bats sampling

Per individual, 2 rectal swabs, 2 oral swabs, and 1 dried blood spot were systematically collected when possible. One swab of each specimen was stored in VTM, the other in TRIzol. Opportunistically, urine was collected and stored in VTM, and fresh feces were collected from the bat bag or directly from the bat and stored in both VTM and ethanol 70% (for *Rhinolophus* species only) to be shared for microbiome analysis. Overall, 1,070 specimens were collected (Table 5).

Table 5: Frequency of specimen collected.

Specimen	VTM	TRIzol	Ethanol 70%	None	Total
Oral swab	184	185	-	-	369
Rectal swab	185	185	-	-	370
Dried blood spot	-	-	-	185	185
Serum	-	-	-	-	-
Feces	59	-	55	-	114
Urine	32	-	-	-	32
Total	460	370	55	185	**1,070**

4. Environmental sampling

4.1. Bat guano collection

Individual bat guanos were collected in one cave in Ka Ngoark on 07/12/2023) inside the cave on top of the hill where nectar and insectivorous bats are co-roosting. In total, 100 bat feces were collected and stored in viral transport medium (VTM) completed with ceramic beads (for homogenization) and 20 additional feces stored in Ethanol (EtOH) 70%. On 09/12/2023, 110 individual guanos from the nectar bats cave on top of Chab Pleurng were collected in VTM and ceramic beads, and 13 additional guanos were stored individually in EtOH 70%.

4.2. Air sampling

A total of 18 air samples was collected using both Aerocollect ("small air sampler", n=12) and Thermofisher ("big air sampler", n=6) instruments. Small air samplers were deployed during sampling sessions (air sampler were displayed on the table during bat sampling) during five consecutive nights of sampling (from 5 to 9/12/2023). Sampling using Thermofisher instruments occurred inside bat caves in Chhgnauk (ground cave and "monk"cave) and Ka Ngoark (ground cave) hills over 3h of sampling. Sampling was doubled each time to allow collection of air samples that were stored 1) in VTM for virus screening, 2) dry for ULiège team to assess feasibility of species identification through metagenomics approaches.

4.3. Environmental sampling from ULiège

A total of 100 small mammal hair traps were set up following table A schedule. Briefly, two diameter sized traps (80 and 50 mm) were set up along lines close to bat trapping areas (see table 6: location of trapping per site). Hair traps were set up with sweet potato baits and left for three consecutive nights. Overall, we potentially had 27/100 positive traps after microscopic inspection, meaning the potential presence of small mammal hair on 27 traps (see details per site in Table 7). To validate our results, further genetic investigation is needed. However, we expected a lower positive rate (10%), reflective of classical rodent trapping methods so we are very optimistic about this set up. Discussions were held potentially indicating the need to optimize methods, specifically: bait types, logistics, time of trap deployment. Bait types were tested on IPC grounds after the field mission and results still need to be assessed, while logistics are already being prepared (use of GPS points, strict protocol). Finally, the potential need for a team of at least two people to leave 3-4 days before the bat sampling team to set up hair traps longer has already been discussed. It will be tested with the return of ULiege team for the April session.

Table 6: eDNA sampling schedule.

Hills/Time	04-12-23	05-12-23	06-12-23	07-12-23	08-12-23	09-12-23	10-12-23
Chhgnauk		Set up AM + soil + leaf swabs			Collection AM		
Ka Ngoark		Set up PM	soil + leaf swabs		Collection PM		
Chab Pleurng			Set up PM	soil + leafswabs		Collection PM	

Table 7: Hair trapping success.

Hills/Traps	Bait eaten		Presence of hair		Total	Potential capture rate
	No	Yes	No	Yes		
Chhgnauk	30	10	25	15	40	38%
Ka Ngoark	30	8	32	5	38	13%
Chab Pleurng	15	5	13	7	20	35%

Regarding environmental samples, 30 leaf swabs were collected per site (nine + one blank per bat trapping location), as well as three soil samples per site (and one blank sample, table 8). Using a 10m2 grid line (close to net position), nine subsamples of soil were randomly collected into one pooled sample, while a collector tube was left open during sampling to be used as blank. In total, 11 "soil mammals" kits from Nature Metrics were used. In the same area, three random leaves at three different heights (ground level – shrubs level – tree level) were swabbed for 2 minutes and then stored in 70% ethanol. Pictures of sites and leaves were taken each time. Sampling was straightforward and went smoothly (Figure 2).

Table 8: eDNA samples collected. In parenthesis are the number of blank samples.

Hills/Samples	Soil	Leaf swabs
Chhgnauk	4 (1)	30 (3)
Ka Ngoark	3 (1)	30 (3)
Chab Pleurng	4 (1)	30 (3)

Figure 2: (1) Example of hair trap set up, (2) Leaf swabbing, (3) Picture of trapping location at Chab Pleung (forest close to Pagoda).

5. Vectors and ectoparasites collection

5.1. Mosquito and Phlebotomine trapping

CDC Light trap was chosen to study in this mission and every 24 hours we collect the mosquitoes from each trap over 5 days collection. The trap was set up at 5 different places by 2 traps was set up in front of the cave, 2 in the forest around the cave and one trap in forest behind our station (Figure 3).

We change the net and battery every 24 hours for 6 Days. The aim being to assess the Culicidae diversity, the collecting time can be variable, according to the movements of the Virology team. We keep everything (all insects) by each trap and keep in the petri dish with parafilm it well and store in the cool box with ice.

Figure 3: Location of the CDC light traps.

In four following days of trapping, all Diptera were sorted and kept. Only 1 family (Culicidae) we identified to the species but some of them we can't ID because it loses some parts like scales leg wing etc. and stored in -200C (Table 8). Sand fly we sorted and stored in Ethanol 70% in lab (Table 9).

Table 8: Culicidae checklist by each trap.

Species	LT 1	LT 2	LT 4	LT 5	Total
Anopheles separatus				2	2
Anopheles sp.			1		1
Culex brevipalpis		1		2	3
Culex gelidus	2				2
Culex malayi	1			1	2
Culex sp.	3	1	1	1	6
Culex vishnui	1	1			2
Mansonia sp.	2				2
Uranotaenia nivipleura				1	1
Uranotenia bicolor		1			1
Uranotenia rampe		1			1
Total	9	5	2	7	23

Table 9: Sand fly by each trap.

	LT 1	LT 2	LT 4	LT 5	Total
Phlebotomine	874	750	93	40	1757

Note:

- LT1 and LT2 were set up in front of bat cave.
- LT3 and LT4 were set up in the forest around the bat cave.
- LT 5 was set up close to the sleeping station.

5.2. Bat ectoparasites collection

The idea is to collect all ectoparasites on bat. Bats should be sampled randomly, 10 bats per species will be collected. This process will take as long as it is required. By manual technique the ecto-parasite was collected with forceps and keep in Eppendorf tube which filled of ethanol 70%.

During the mission, 186 bats belonging to16 species within 8 genera were sampled. 70% of the sampled bats hosted ectoparasites (Table 10). A total of 415 specimens of ectoparasites belonging to 8 families were sampled. Details of the captures are given in tables 11, 12 and 13.

One specimen of *Brachytarsina modesta* was collected on *Eonycteris speleae*. However, this batflies is specific to microchiropteran bats, especially on rhinolophid bats. This association is therefore considered as accidental (red number in the following tables).

Many specimens of *Eucampsipoda inermis* (Nycteribiidae) have been collected directly on the wall or on the rocks on the ground in a cave sheltering several thousand bats (top of the hill, Chab Pleurng). All of these specimens were pregnant females and were probably looking for a site to deposit their larva. These observations support the fact that only females leave their host's body solely to give birth. It was probably the first time that this species was observed directly in the bat's habitat. This cave was very rich in biodiversity because millions of larvae were in the guano, as well as many specimens of *Gnathoncus vietnamicus* (Histeridae, species known only from two specimens in the world) and a new species of *Gebieniella* (Tenebrionidae, Steonisini).

Remarks: Taxonomic changes were not considered in previous reports. The genus *Stylidia* is now *Phthiridium*. Thus, *Stylidia fraterna* becomes *Phthiridium fraternum*, *S. caudata* becomes *P. caudatum* and *S. ornata* becomes *P. ornatum*. The subgenus *Leptocyclopodia* has been upgraded to genus status. Thus, *Cyclopodia* (*Leptocyclopodia*) *ferrarii* now becomes *Leptocyclopodia ferrarii*.

Table 10: 186 bat individuals were selected to check all ectoparasites.

Bat species	Ectoparasites		Total
	Absent	Present	
Cynopterus sphinx	1	2	3
Cynopterus brachyotis	5	1	6
Cynopterus sp.		2	2
Eonycteris speleae		1	1
Eonycteris sp.		1	1
Hipposideros armiger		1	1
Hipposideros sp.	1		1
Lyroderma lyra		1	1
Megaderma spasma	4		4
Rhinolophus malayanus	3	2	5
Rhinolophus pusillus	1	3	4
Rhinolophus shameli	17	100	117
Rhinolophus stheno (TBC)	1		1
Taphozous melanopogon	21	15	36
Taphozous sp.	1	1	2
Roussetus sp.	1		1
Total	56	130	186

Table 11: Species richness of the ectoparasites for the different sites of capture and bat species during the mission.

Bat species	Site			Total
	Chab Pleurng	Chhngauk	Kar Ngaork	
Cynopterus sphinx			1	1
Cynopterus brachyotis	1			1
Cynopterus sp.		1		1
Eonycteris speleae	2			2
Eonycteris sp.		1		1
Hipposideros armiger			1	1
Lyroderma lyra			1	1
Rhinolophus malayanus			3	3
Rhinolophus pusillus	2		2	4
Rhinolophus shameli	3	3	3	9
Taphozous melanopogon		1	2	3
Taphozous sp.			1	1
Total	8	6	14	28

Table 12: All ectoparasites with their hosts bats collected during the mission.

Bat species	Ticks & Mites	Fleas	Bat fly		Bat bugs	Total
		Ischnopsyllidae	Nycteribiidae	Streblidae	Polyctenidae	
Cynopterus sphinx			2			2
Cynopterus brachyotis			1			1
Cynopterus sp.			3			3
Eonycteris speleae	3			**(1)**		3
Eonycteris sp.	2	2				4
Hipposideros armiger	1					1
Lyroderma lyra				28		28
Rhinolophus pusillus				6		6
Rhinolophus shameli	3		38	290		
Rhinolophus malayanus			1	2		3
Taphozous melanopogon	12			10	2	24
Taphozous sp.	1					1
Roussetus amplexicaudatus cave			8			8
Total	22	2	53	336	2	415

Table 13: Abundance of Streblidae and Nycteribiidae from the different host bat species during the mission.

Bat species	Nycteribiidae			Streblidae				Total
	P. fraternum	*L. ferrarii*	*E. inermis*	*B. modesta*	*B. cucullata*	*R. pseudopagodarum*	*R. lobulata*	
R. shameli	38			44		246		328
R. pusillus				3		3		6
R. malayanus	1			1		1		3
T. melanopogon					10			10
C. sphinx		2						2
C. brachyotis		1						1
Cynopterus sp.		3						3
E. speleae				**-1**				0
L. lyra							28	28
R. amplexicaudatus (cave)			8					8
Total	39	6	8	48	10	250	28	**389**

6. Bat acoustic protocol

One of the objectives of this field mission was to review and implement a protocol for bats acoustic study in order to ass the spatial distribution of bats, focusing on *Rhinolophus shameli* species. Eight song meter mini acoustic recorder devices (Virology Unit), 2 song meter SM4 (CIRAD), and 1 song meter SM4 from Neil Furey were available for acoustic recordings. The team deployed the 10 devices over 5 nights in total, and in different landscape types: dry dipterocarp forest (DDF), plantation, field, village. Paired deployment was assessed to account for the presence (or not) of water supply nearby (stream, pond, river…).

The figure 4 shows the different locations where the acoustics devices have been deployed during this field work.

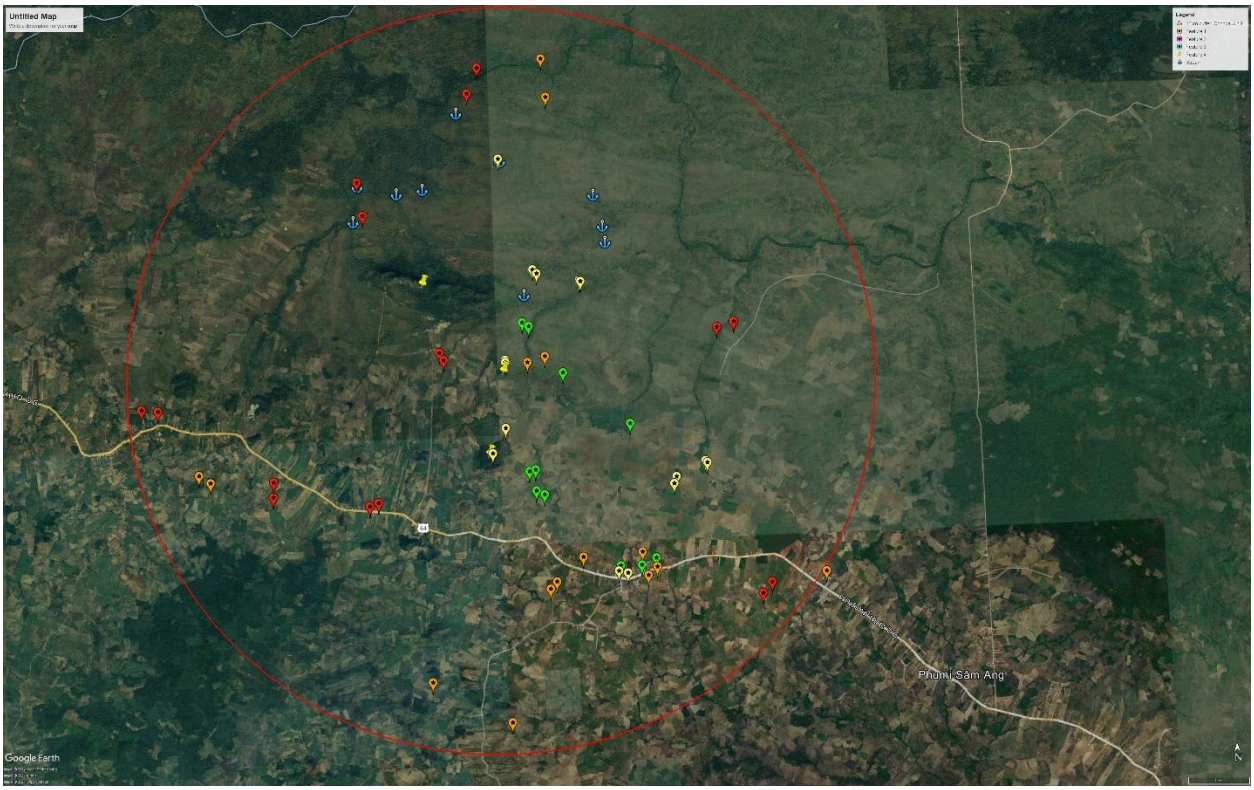

Figure 4: Map of the deployment of bat acoustic devices. Red circle: buffer area of 10km around Chhgnauk hill; yellow mark: “static” location; green mark: “mobile” location from May 2023; orange mark: “mobile” location from June 2023; red mark: “mobile”location from December 2023; Anchor mark: water source.

7. Camera trap protocol

Camera traps were set up at the entrance of selected caves to assess visit of the cave by human and other animals (domestic and wild). They were programmed to be activated only upon detection of movement, and to capture one video of 10 sec and 3 pictures each time. Cameras should stay in place for at least a year.

At the ground cave of Chhgnauk hill, 2 cameras were set up: one inside the cave, capturing movements inside the cave; one outside, facing the main entrance. The camera installed inside the ground cave at Ka Ngoark hill was stolen. The team has been informed by local guide in October 2023. No more camera

is thus installed in Ka Ngoark and the team is reviewing options to set up a new one there or focus only in Chhgnauk hill.

The change of batteries and SD cards is running smoothly.

8. To be improved/changed

- Departure from IPC on the first day of mission should be advanced a bit to ensure arrival at Chhgnauk hill at least 1h prior departing for capture.
- Biosafety reminders (how to put on/off PPE…) should be printed out and made available to everyone on site.
- Feces to be stored in EtoH during sampling sessions should be collected for all bat species, and not only Rhinolophus species during the next missions.
- Collection of ectoparasites should be done with the ID group and no more during sampling, to avoid losing ectoparasites.
- Argasidae and bat flies were collected on the rock walls inside the cave at the top of Chab Pleurng. To study bat ectoparasites ecology, it is recommended to stay longer in that cave during next field work.
- It is recommended to try 2 or 3 BG and 5LT during next mission.