



De nouveaux coronavirus de chauve-souris similaires à SARS-CoV-2 éclairent l'origine de la pandémie de COVID-19

Sarah Temmam, Khamsing Vongphayloth, Eduard Baquero Salazar, Sandie Munier, Massimiliano Bonomi, Béatrice Regnault, Bounsavane Douangboubpha, Yasaman Karami, Delphine Chrétien, Daosavanh Sanamxay, et al.

► To cite this version:

Sarah Temmam, Khamsing Vongphayloth, Eduard Baquero Salazar, Sandie Munier, Massimiliano Bonomi, et al.. De nouveaux coronavirus de chauve-souris similaires à SARS-CoV-2 éclairent l'origine de la pandémie de COVID-19. Journées Francophones de Virologie, Apr 2022, Strasbourg, France. [pasteur-03663296](#)

HAL Id: [pasteur-03663296](#)

<https://pasteur.hal.science/pasteur-03663296>

Submitted on 10 May 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



De nouveaux coronavirus de chauve-souris similaires à SARS-CoV-2 éclairent l'origine de la pandémie de COVID-19.

Sarah Temmam^{1,2,*}, Khamsing Vongphayloth^{3,*}, Eduard Baquero Salazar^{4,*}, Sandie Munier^{5,*}, Massimiliano Bonomi^{6,*}, Béatrice Regnault^{1,2}, Bounsavane Douangboubpha⁷, Yasaman Karami⁶, Delphine Chrétien^{1,2}, Daosavanh Sanamxay⁷, Vilakhan Xayaphet⁷, Phetphoumin Paphaphanh⁷, Vincent Lacoste³, Somphavanah Somlor³, Khaithong Lakeomany³, Nothasin Phommavanh³, Philippe Pérot^{1,2}, Océane Dehan^{5,8}, Faustine Amara⁵, Flora Donati^{5,8}, Thomas Bigot^{1,9}, Michael Nilges⁶, Félix A. Rey⁴, Sylvie van der Werf^{5,8}, Paul T. Brey³, Marc Eloit^{1,2,10,+}

¹ Institut Pasteur, Université de Paris, Pathogen Discovery Laboratory, 25-28 rue du Dr. Roux, 75015, Paris, France.

² Institut Pasteur, The OIE Collaborating Center for the detection and identification in humans of emerging animal pathogens, 25-28 rue du Dr. Roux, 75015, Paris, France

³ Institut Pasteur du Laos, Samsenthai Road, Ban Kao-Gnot, Sisattanak District, 3560 Vientiane, Lao PDR

⁴ Institut Pasteur, Structural Virology Unit, CNRS UMR 3569, 25-28 rue du Dr. Roux, 75015, Paris, France.

⁵ Institut Pasteur, Université de Paris, CNRS UMR 3569, Molecular Genetics of RNA Viruses Unit, Paris, France

⁶ Institut Pasteur, Université de Paris, CNRS UMR 3528, Structural Bioinformatics Unit, Paris, France

⁷ Faculty of Environmental Sciences, National University of Laos, Dong Dok Campus, P.O. Box: 7322, Xaythany District, Vientiane Capital, Lao PDR

⁸ Institut Pasteur, National Reference Center for Respiratory Viruses, 25-28 rue du Dr. Roux, 75015, Paris, France.

⁹ Institut Pasteur, Bioinformatic and Biostatistic Hub - Computational Biology Department, 25-28 rue du Dr. Roux, 75015, Paris, France.

¹⁰ Ecole Nationale Vétérinaire d'Alfort, University of Paris-Est, 94704 Maisons-Alfort, France

* co-first authors

+ corresponding author

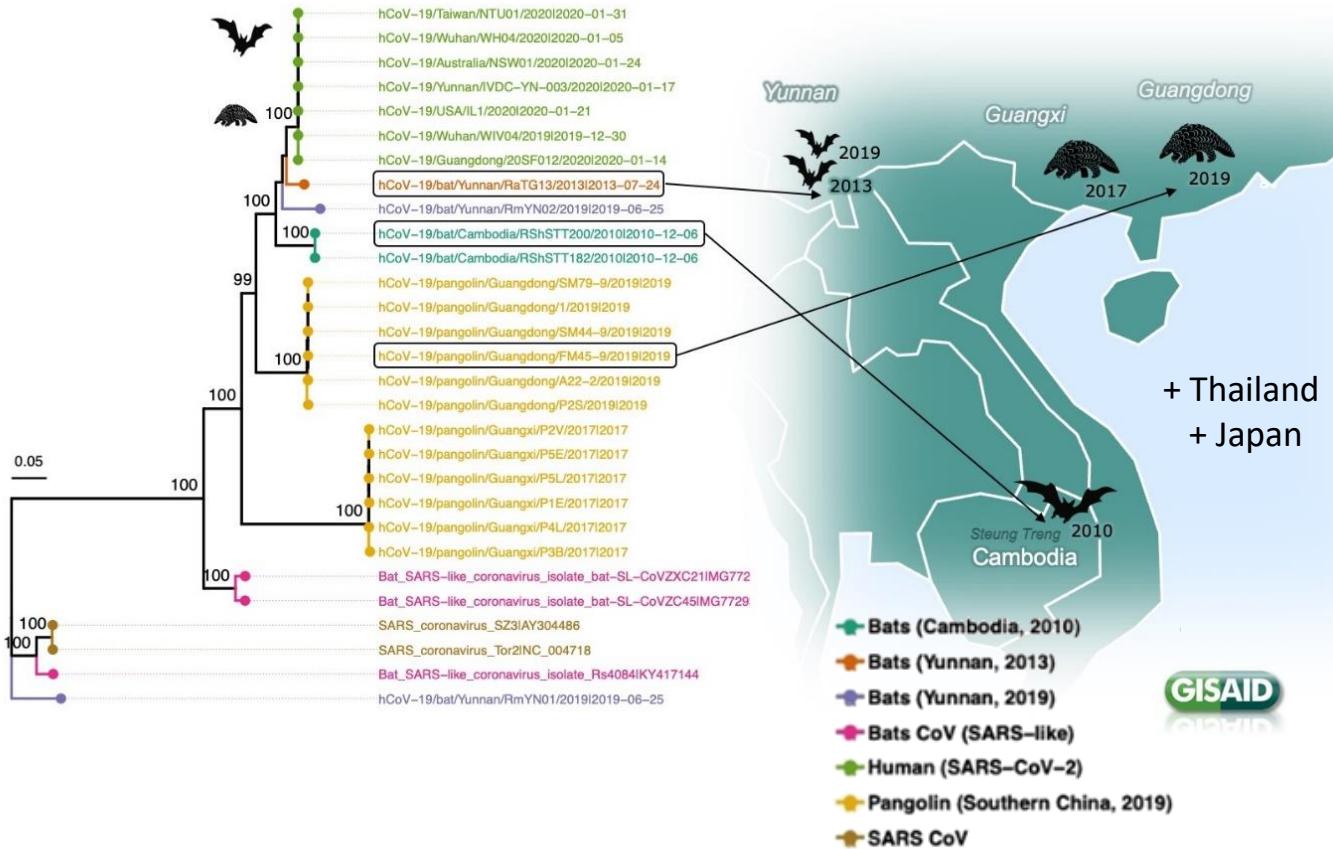
Context

- Numerous SARS-CoV-2-related viruses identified in **bats & pangolins**
- First cases detected in **Wuhan** (Huanan seafood market & elsewhere)
- First case 8th December 2019

Worobey M. 2021 Science



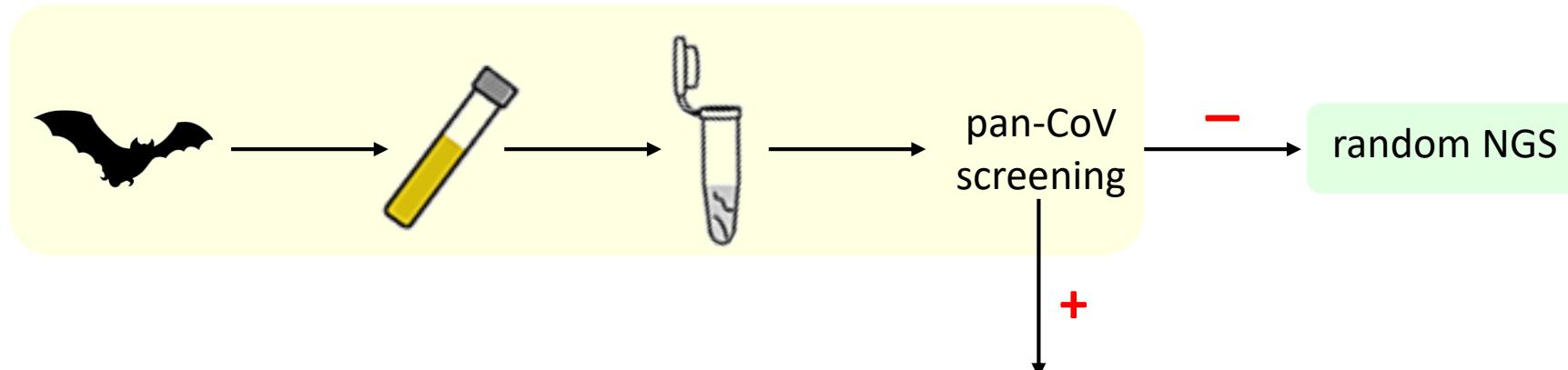
- Animal reservoir?
- Intermediate host?
- Routes of introduction into human populations?
- Silent circulation in humans before detection?



Objectives

- To improve the understanding of the **origins, natural history and dissemination** of SARS-CoV-2 and SARS-like coronaviruses **in their natural environment**
 - Identify natural reservoir(s) of SARS-CoV-2
 - Decipher interspecies transmission (bat-to-bat, bat-to-mammals, bat-to-humans)
 - To detect **past coronavirus infections** in humans exposed to animal reservoirs
 - Identify early circulation of SARS-CoV-2 or SARS-CoV-2-like viruses before global spread
- Ecological factors
that lead to
SARS-CoV-2
emergence**

Workflow & consortium



Characterization of the virus

Ecological studies

- Hosts spectrum
- Seasonality
- Persistence in environment
- Virus tropism in bats
- etc.

Full genome sequencing

- Evolution history

Structural modelling

- Interaction with receptor

Pseudotyped lentiviruses

- Virus entry

Virus isolation

- In vivo* studies

Expression structural proteins

- Seroprevalence studies

Ongoing studies

Field work



Khamsing
Vongphayloth
IP-Laos



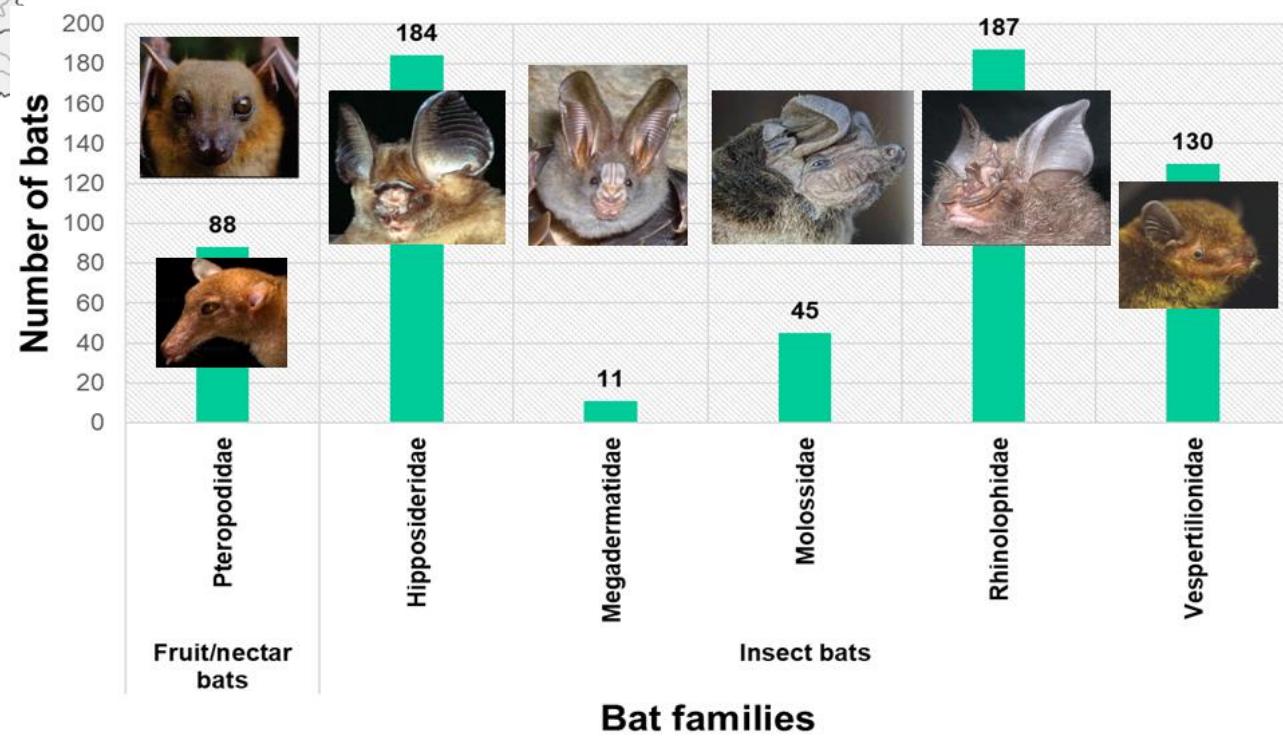
Harp trap



Mist net



- **645 bats**
 - 48 species
 - 6 families
- **Sample types**
 - Oral swabs (608)
 - Rectal swabs (539)
 - Urine (157)
 - Blood (246)



pan-CoV screening



Vincent Lacoste
IP-Laos
(former member)

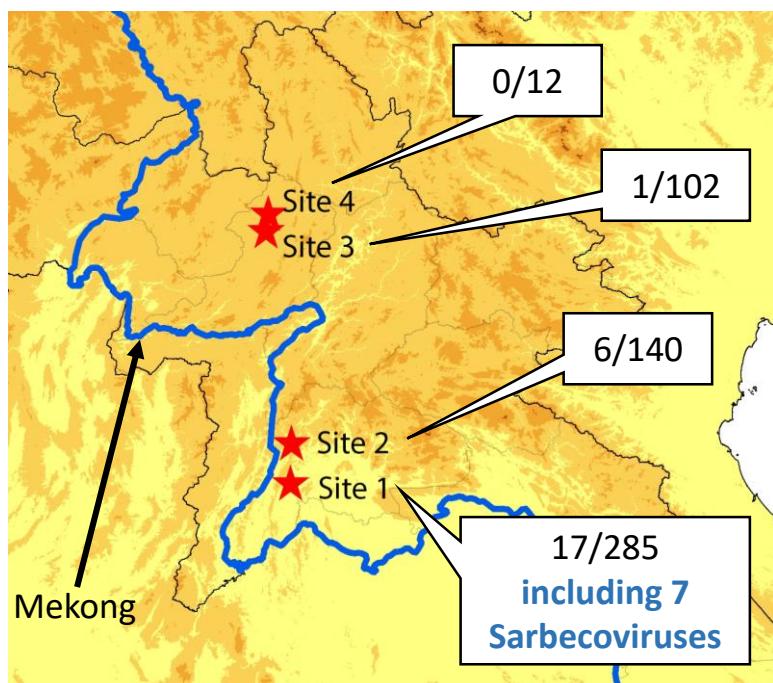
JOURNAL OF VIROLOGY, Dec. 2011, p. 12815–12820
0022-538X/11/\$12.00 doi:10.1128/JVI.05838-11

Copyright © 2011, American Society for Microbiology. All Rights Reserved.

Vol. 85, No. 23

Avian Coronavirus in Wild Aquatic Birds^{†‡}

Daniel K. W. Chu,¹ Connie Y. H. Leung,¹ Martin Gilbert,² Priscilla H. Joyner,² Erica M. Ng,¹ Tsemay M. Tse,¹ Yi Guan,¹ Joseph S. M. Peiris,^{1,3*} and Leo L. M. Poon^{1*}



N=539 rectal swabs

Anal Swab ID	Bat Genus	Bat Species	CoV Genus	CoV Subgenus	Closest sequence	% blastN similarity
BANAL-20-27	<i>Rhinolophus</i>	<i>pusillus</i>	co-infection <i>Alpha/Betacoronavirus</i>	unclassified <i>Decacovirus/Sarbecovirus</i>	Rhinolophus bat coronavirus HKU32 / SARS-CoV-2	80%
BANAL-20-51	<i>Hipposideros</i>	<i>pomona</i>	<i>Alphacoronavirus</i>	<i>Decacovirus</i>	Bat coronavirus HKU10	98%
BANAL-20-52	<i>Rhinolophus</i>	<i>Malayanus</i>	<i>Betacoronavirus</i>	<i>Sarbecovirus</i>	SARS-CoV-2	99%
BANAL-20-103	<i>Rhinolophus</i>	<i>pusillus</i>	<i>Betacoronavirus</i>	<i>Sarbecovirus</i>	SARS-CoV-2	99%
BANAL-20-115	<i>Hipposideros</i>	<i>pomona</i>	<i>Alphacoronavirus</i>	<i>Decacovirus</i>	Bat coronavirus HKU10	98%
BANAL-20-116	<i>Rhinolophus</i>	<i>malayanus</i>	<i>Betacoronavirus</i>	<i>Sarbecovirus</i>	SARS-CoV-2	98%
BANAL-20-178	<i>Hipposideros</i>	<i>pomona</i>	<i>Alphacoronavirus</i>	<i>Decacovirus</i>	Bat coronavirus HKU10	98%
BANAL-20-191	<i>Chaerephon</i>	<i>plicatus</i>	<i>Alphacoronavirus</i>	<i>Pedacovirus</i>	Porcine epidemic diarrhea virus (PEDV)	93%
BANAL-20-197	<i>Chaerephon</i>	<i>plicatus</i>	<i>Alphacoronavirus</i>	<i>Pedacovirus</i>	Porcine epidemic diarrhea virus (PEDV)	93%
BANAL-20-212	<i>Chaerephon</i>	<i>plicatus</i>	<i>Alphacoronavirus</i>	<i>Pedacovirus</i>	Porcine epidemic diarrhea virus (PEDV)	93%
BANAL-20-213	<i>Chaerephon</i>	<i>plicatus</i>	<i>Alphacoronavirus</i>	<i>Pedacovirus</i>	Porcine epidemic diarrhea virus (PEDV)	93%
BANAL-20-236	<i>Rhinolophus</i>	<i>marshalli</i>	<i>Betacoronavirus</i>	<i>Sarbecovirus</i>	SARS-CoV-2	99%
BANAL-20-242	<i>Rhinolophus</i>	<i>malayanus</i>	<i>Betacoronavirus</i>	<i>Sarbecovirus</i>	SARS-CoV-2	99%
BANAL-20-247	<i>Rhinolophus</i>	<i>malayanus</i>	<i>Betacoronavirus</i>	<i>Sarbecovirus</i>	SARS-CoV-2	99%
BANAL-20-251	<i>Hipposideros</i>	<i>khaokhouayensis</i>	<i>Alphacoronavirus</i>	<i>Decacovirus</i>	Bat coronavirus HKU10	97%
BANAL-20-273	<i>Rhinolophus</i>	<i>pusillus</i>	<i>Alphacoronavirus</i>	unclassified <i>Decacovirus</i>	Rhinolophus bat coronavirus HKU32	92%
BANAL-20-289	<i>Rhinolophus</i>	<i>affinis</i>	<i>Alphacoronavirus</i>	<i>Rhinacovirus</i>	Rhinolophus affinis bat coronavirus HKU2-related / Swine acute diarrhea syndrome related coronavirus (SADS-CoV)	98%
BANAL-20-290	<i>Eonycteris</i>	<i>spelaea</i>	<i>Betacoronavirus</i>	<i>Nobecovirus</i>	Rousettus bat coronavirus GCCDC1	99%
BANAL-20-390	<i>Eonycteris</i>	<i>spelaea</i>	<i>Betacoronavirus</i>	<i>Nobecovirus</i>	Rousettus bat coronavirus GCCDC1	97%
BANAL-20-395	<i>Eonycteris</i>	<i>spelaea</i>	<i>Betacoronavirus</i>	<i>Nobecovirus</i>	Rousettus bat coronavirus GCCDC1	99%
BANAL-20-398	<i>Eonycteris</i>	<i>spelaea</i>	<i>Betacoronavirus</i>	<i>Nobecovirus</i>	Rousettus bat coronavirus GCCDC1	99%
BANAL-20-403	<i>Eonycteris</i>	<i>spelaea</i>	<i>Betacoronavirus</i>	<i>Nobecovirus</i>	Rousettus bat coronavirus GCCDC1	99%
BANAL-20-416	<i>Cynopterus</i>	<i>sphinx</i>	<i>Betacoronavirus</i>	<i>Nobecovirus</i>	Rousettus bat coronavirus GCCDC1	99%
BANAL-20-497	<i>Myotis</i>	sp.	<i>Alphacoronavirus</i>	<i>Pedacovirus</i>	Bat alphacoronavirus*	99%

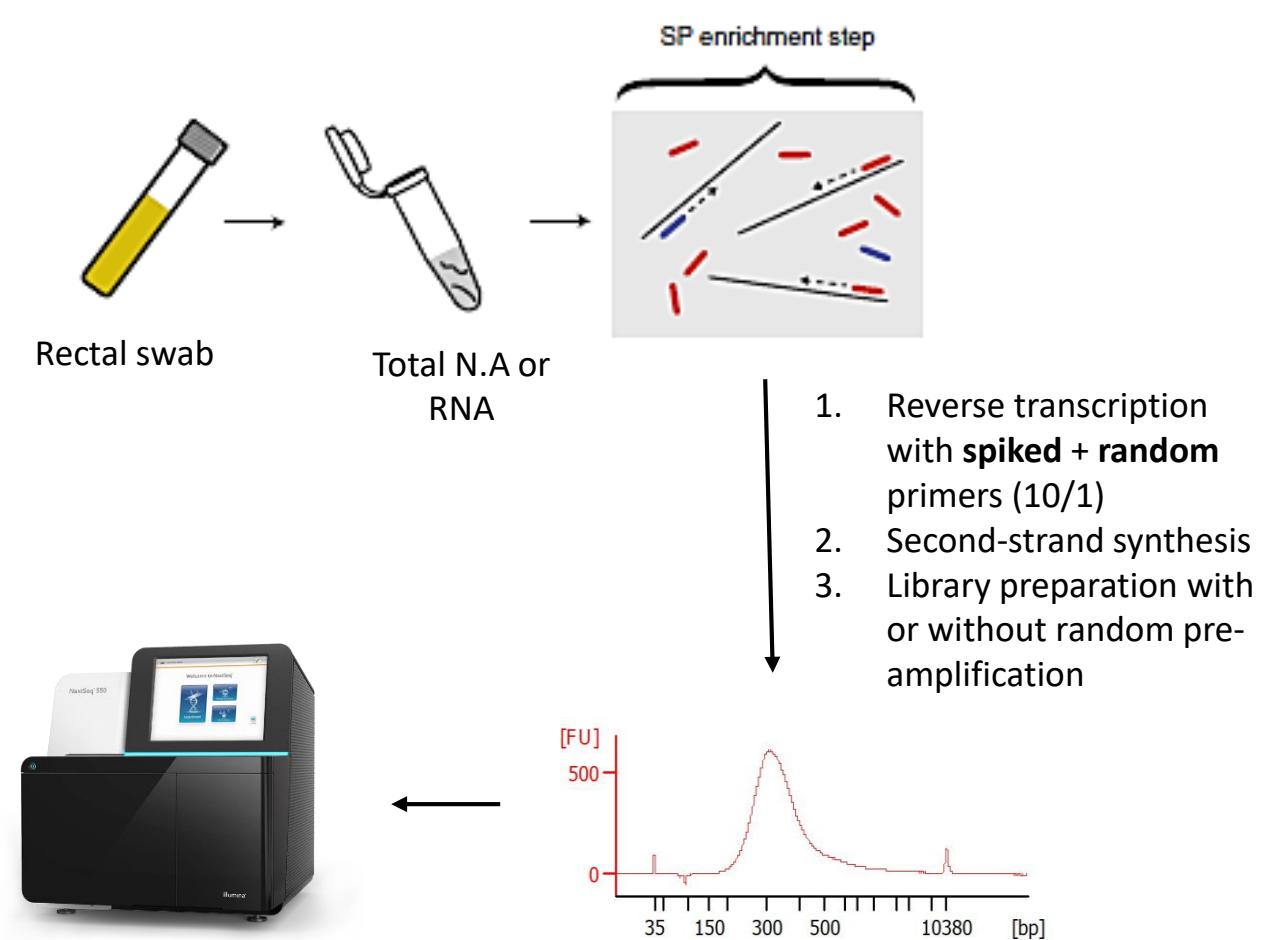
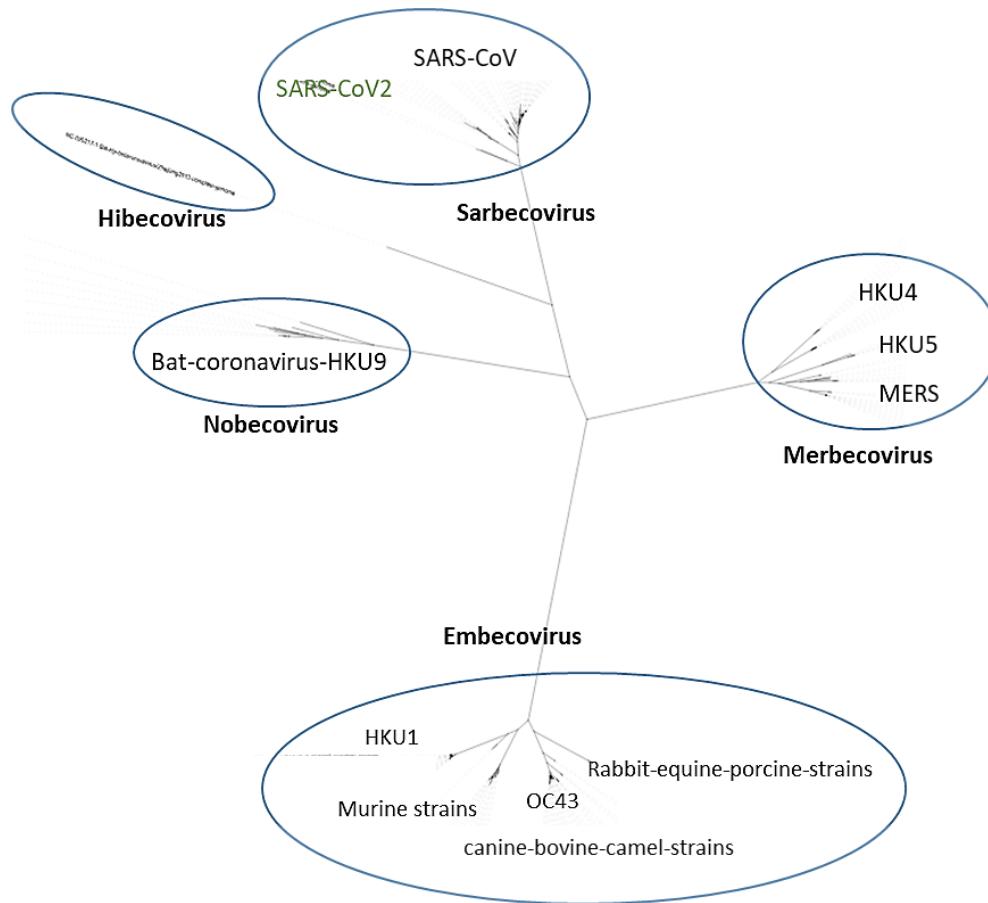
Betacoronavirus enrichment



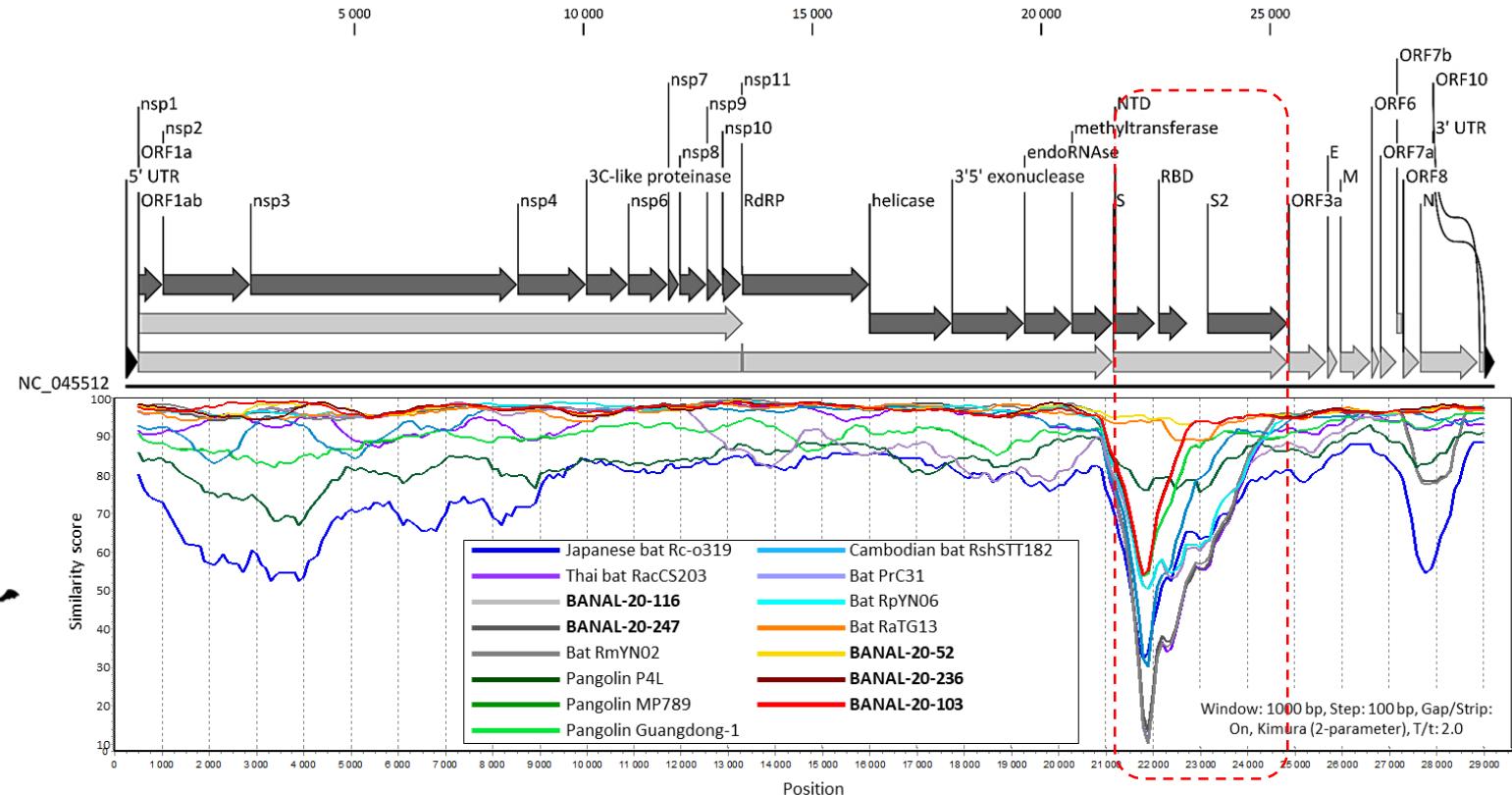
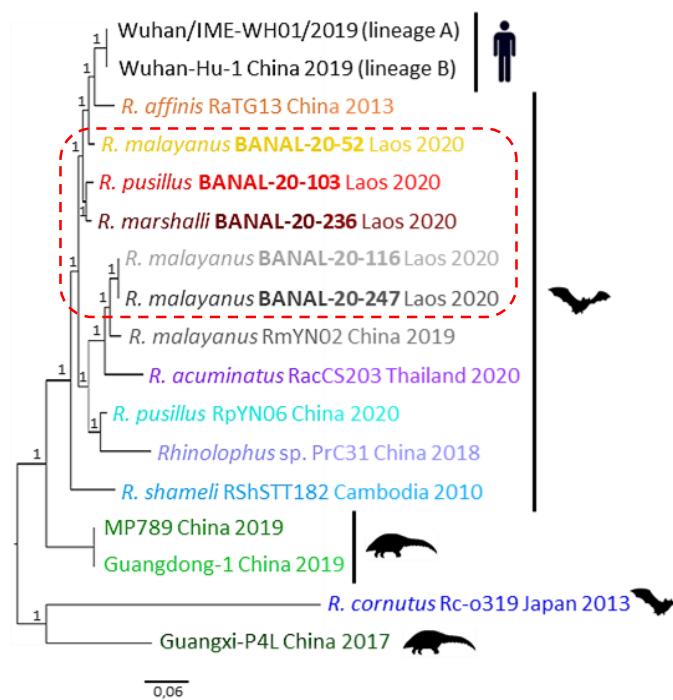
➤ **In-silico probe design** from nucleotide sequence alignment of all *Betacoronavirus* subgenera

Béatrice Regnault
IPP – Pathogen
Discovery lab.

- 13-mer primers pan-generic or specific to each subgenus (MSSPE method) Deng X. et al., 2020 Nat. Microbiol. doi: 10.1038/s41564-019-0637-9
- 432 primers



Evolution history of bat CoVs



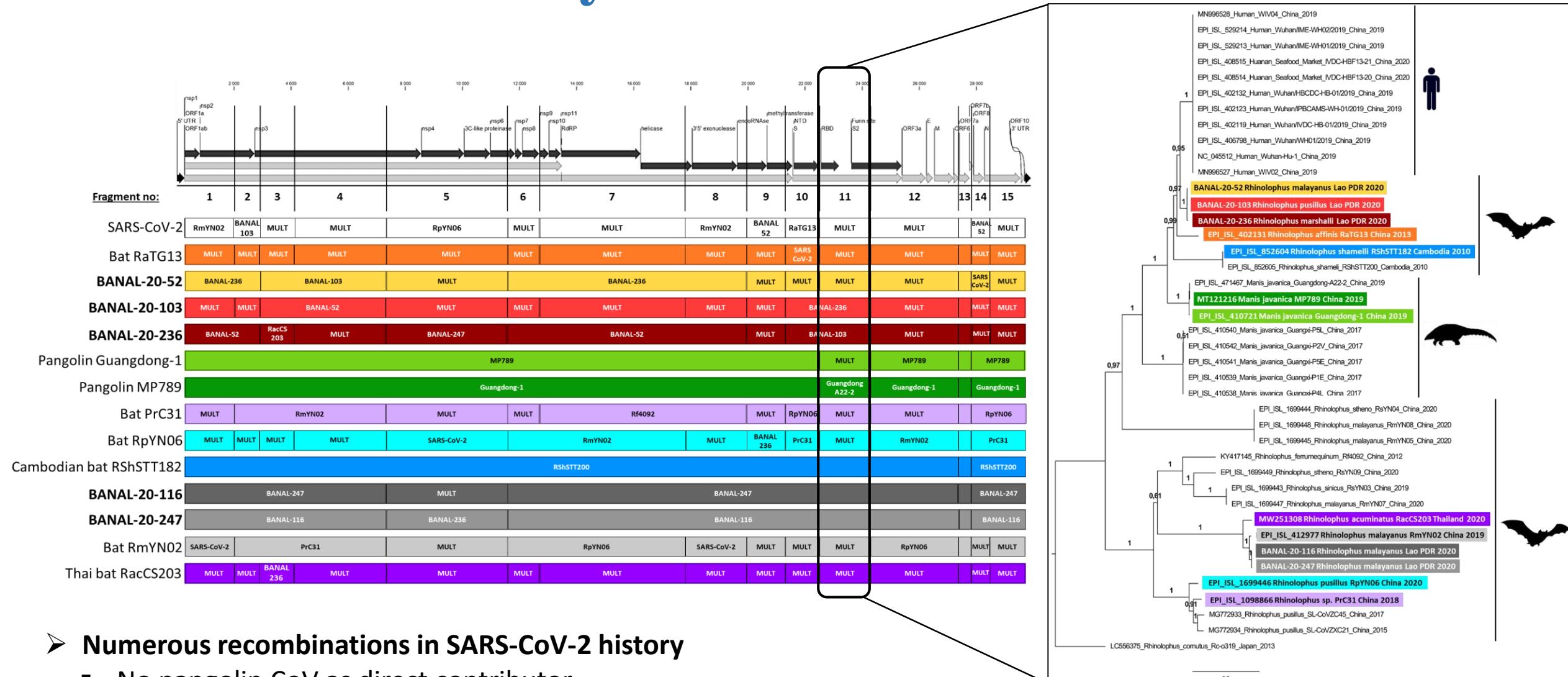
➤ At the whole genome level

- BANAL viruses close to SARS-CoV-2 early strains
- High degree of conservation between CoVs carried by different *Rhinolophus* species living in sympatry

➤ Similarity along the genome

- BANAL viruses present >95% nt identity along the genome, BANAL-52 the closest known to date
- In the spike, BANAL viruses closer to SARS-CoV-2 than bat and pangolin CoVs
- No furin site

Evolution history of bat CoVs



➤ Numerous recombinations in SARS-CoV-2 history

- No pangolin CoV as direct contributor
- One / multiple ancestors depending on the fragment of genome
- In the spike: RaTG13 CoV for the NTD, BANAL CoVs for RBD and N-terminal part of S2 (including the furin site)

Bat CoV RBD highly similar to SARS-CoV-2

SARS-CoV-2 lineage A	319	RVQPTESIVRFPNITNLCPFGEVFNA TRFASVYAWN RKRISNCVAD YSVLYNSASFSTFKCYGVSPKTLNDLCFTNVYADSFVIRGDEVR QIA PGQTGKIA D NYKLPDDFT	430
SARS-CoV-2 lineage B	319	RVQPTESIVRFPNITNLCPFGEVFNA TRFASVYAWN RKRISNCVAD YSVLYNSASFSTFKCYGVSPKTLNDLCFTNVYADSFVIRGDEVR QIA PGQTGKIA D NYKLPDDFT	430
BANAL-20-52	319T.....T.....V.....	430
Bat RaTG13	319D.....T.....T.....	430
Pangolin P4L	317I.....SK.....T.....VK.....V.....	428
BANAL-20-103	315D.....T.....T.....V.....	426
BANAL-20-236	315D.....T.....T.....V.....	426
Pangolin Guangdong-1	315T.....T.....V.....R.....	426
Pangolin MP789	315T.....T.....V.....R.....	426
Bat PrC31	315Q.V.....V.....HK.....P.....E.TK..D.I.....T.F.....T.....S.I.....S.....T.L.FS.....V.....V.....	426
Bat RpYN06	315Q.V.....V.....HN.....P.....E.TK..D.I.....T.F.....T.....S.I.....S.....T.L.FS.....V.....V.....	426
Cambodian bat RShSTT182	306	TS..TQV.....T.....R.....TT.....V.....	M 417
BANAL-20-116	298	.L STEV.....F.....DK.....PN.....Q.TK..D.I.....T.....T.....S.I.....S.....T.L.FS.....E.....V.....	409
BANAL-20-247	298	.L STEV.....F.....DK.....PN.....Q.TK..D.I.....T.....T.....S.I.....S.....T.L.FS.....E.....V.....	409
Bat RmYN02	298	.IL STEV.....F.....DK.....PN.....Q.TK..D.I.....T.....T.....S.I.....S.....T.L.FS.....E.....V.....	409
Thai bat RacCS203	298	.L STEV.....F.....DK.....PN.....Q.TK..D.I.....T.....T.....S.I.....S.....T.L.FS.....E.....V.....	409
Japanese bat Rc-o319	293QDTV.....S.....T.....R.....T.....Q.....S.....V.....	404

➤ Alignment of the RBD region

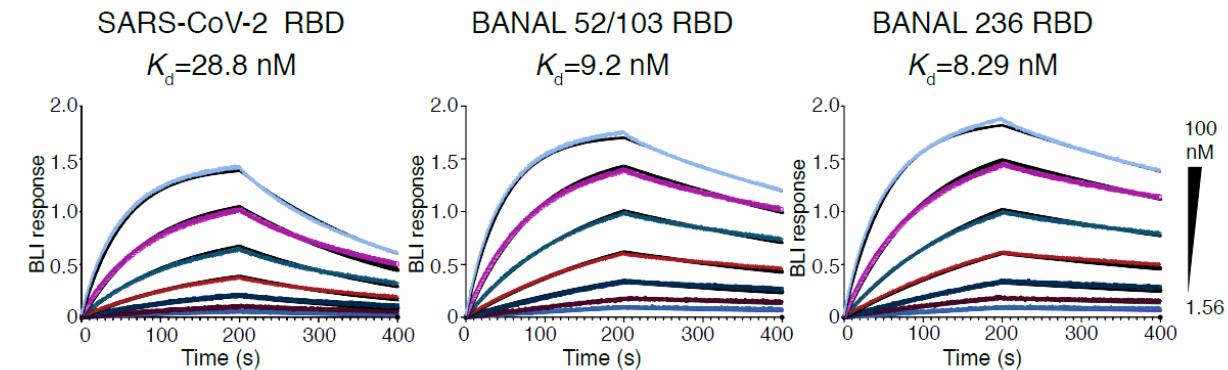
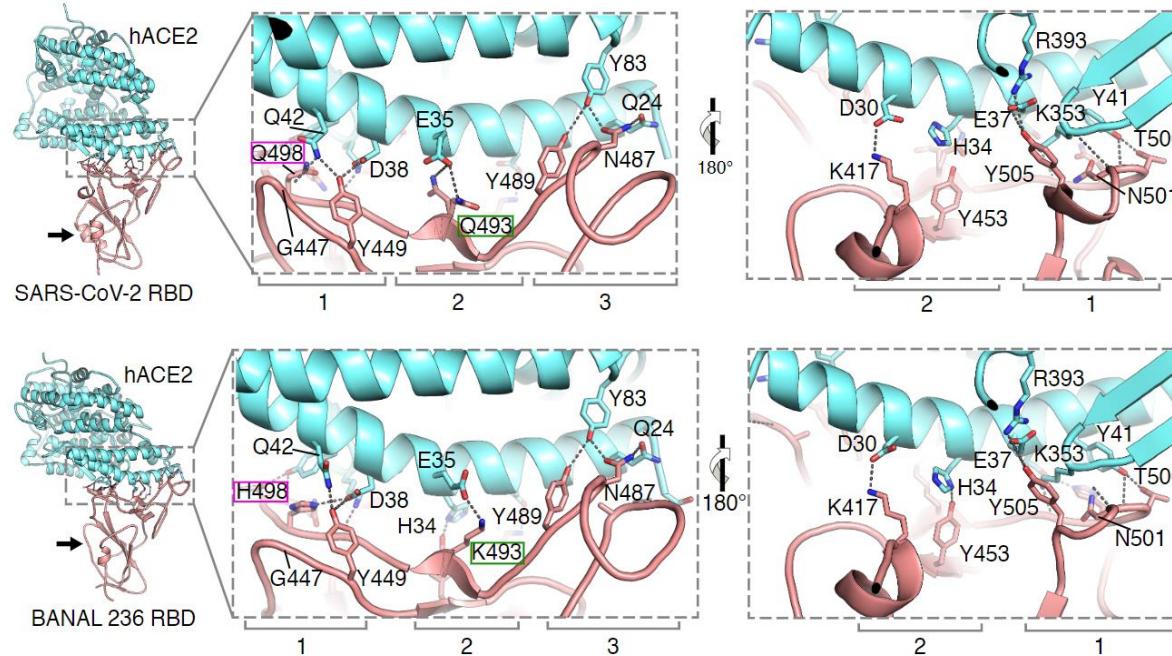
- BANAL-52 (*R. malayanus*) closer to SARS-CoV-2 than any bat / pangolin CoVs
 - 15-16/17 aa residues interacting with hACE2 receptor conserved between BANAL viruses and SARS-CoV-2 (13/17 for Cambodian bat *R. shameli* and 11/17 for Chinese bat *R. affinis* RaTG13)

Insights into RBD/hACE2 complex



Eduard Baquero-Salazar
IPP – Structural Virology

Massimiliano Bonomi
IPP – Structural Bioinformatics

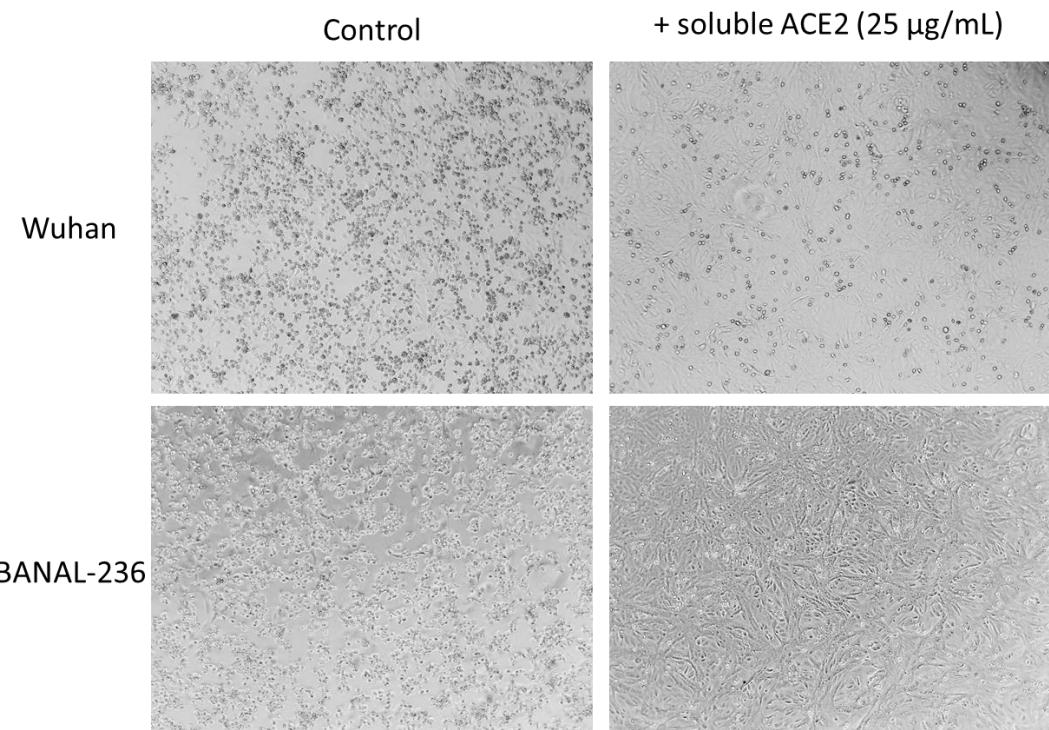
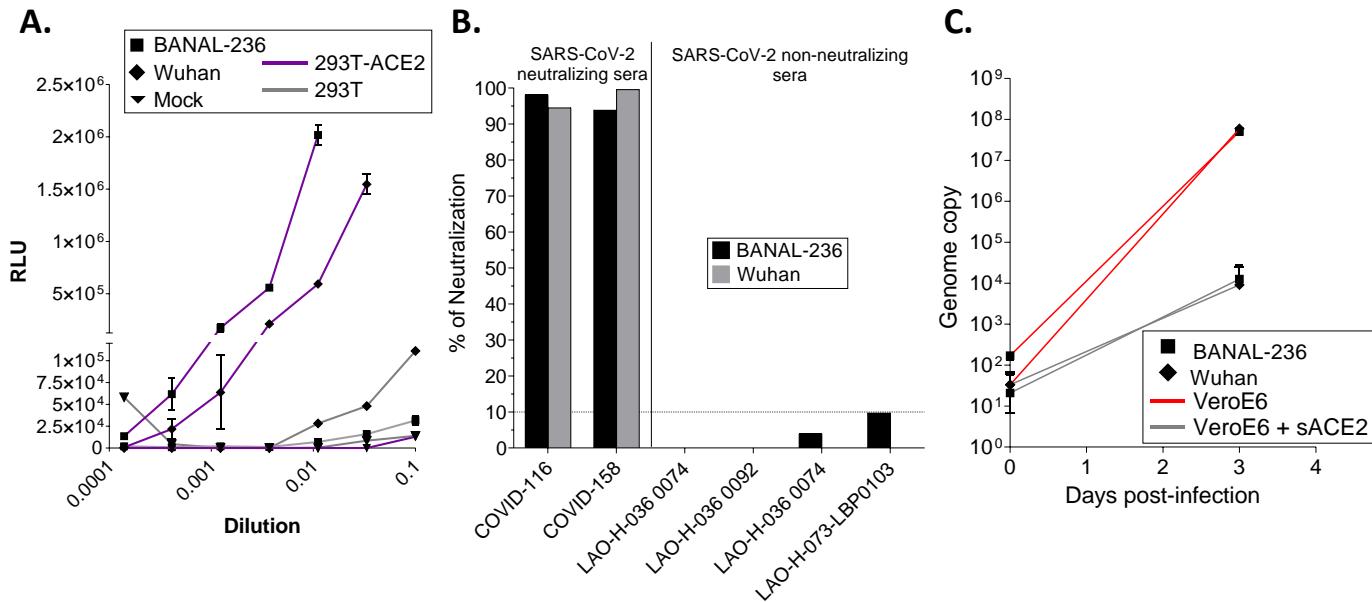


- Determination of the crystal structure of BANAL RBDs
- Effects of mutations in RBD-hACE2 interactions
 - BANAL mutation $H498Q$ disrupt transient hydrogen bonds but is known to increase affinity of SARS-CoV-2 RBD for hACE2 Huang et al., 2021 EBioMedicine
 - BANAL mutations $K493Q \rightarrow$ 2 additional salt bridges formed between BANAL-236 RBD & ACE2 → possible \nearrow affinity
- Biolayer interferometry
 - K_d BANAL viruses << SARS-CoV-2 $\longrightarrow \nearrow$ affinity of BANAL viruses compared to SARS-CoV-2 Wuhan strain

Cell entry through ACE2-dependent pathway



Sandie Munier
IPP – Molecular
Genetics of RNA
Viruses

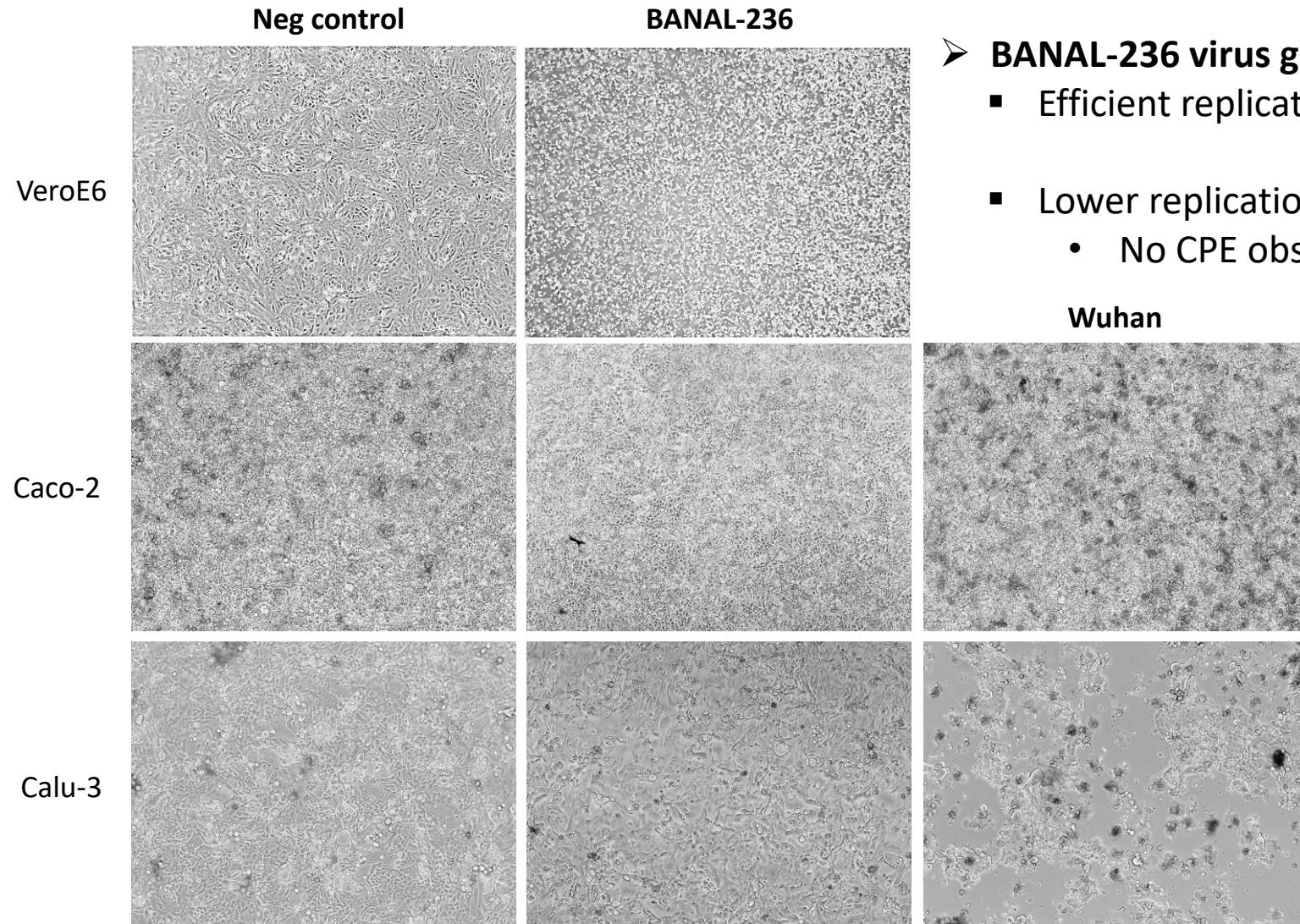


- Lentiviral pseudoparticles expressing Wuhan or BANAL-236 wild type spikes
 - Entry in 293T cells expressing hACE2
 - Entry blocked by SARS-CoV-2 neutralizing sera
- BANAL-236 virus isolation
 - Infection VeroE6 expressing endogenous ACE2
 - Infection blocked by soluble ACE2 ($-3,6 \log_{10}$) similar to Wuhan ($-3,8 \log_{10}$)

Virus isolation

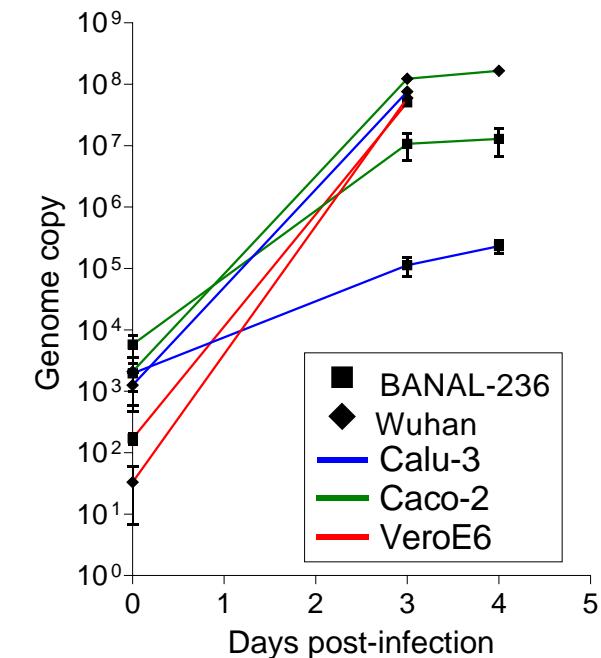


Flora Donati
IPP – Molecular
Genetics of RNA
Viruses



➤ BANAL-236 virus growth

- Efficient replication in VeroE6, similar yield compared to Wuhan
- Lower replication in human cells expressing endogenous ACE2
 - No CPE observed compared to Wuhan



Ongoing studies

Virus
isolation
↓
In vivo
studies

Expression
structural proteins
↓
Seroprevalence
studies

➤ Pathogenicity in animal model (mice + primates)

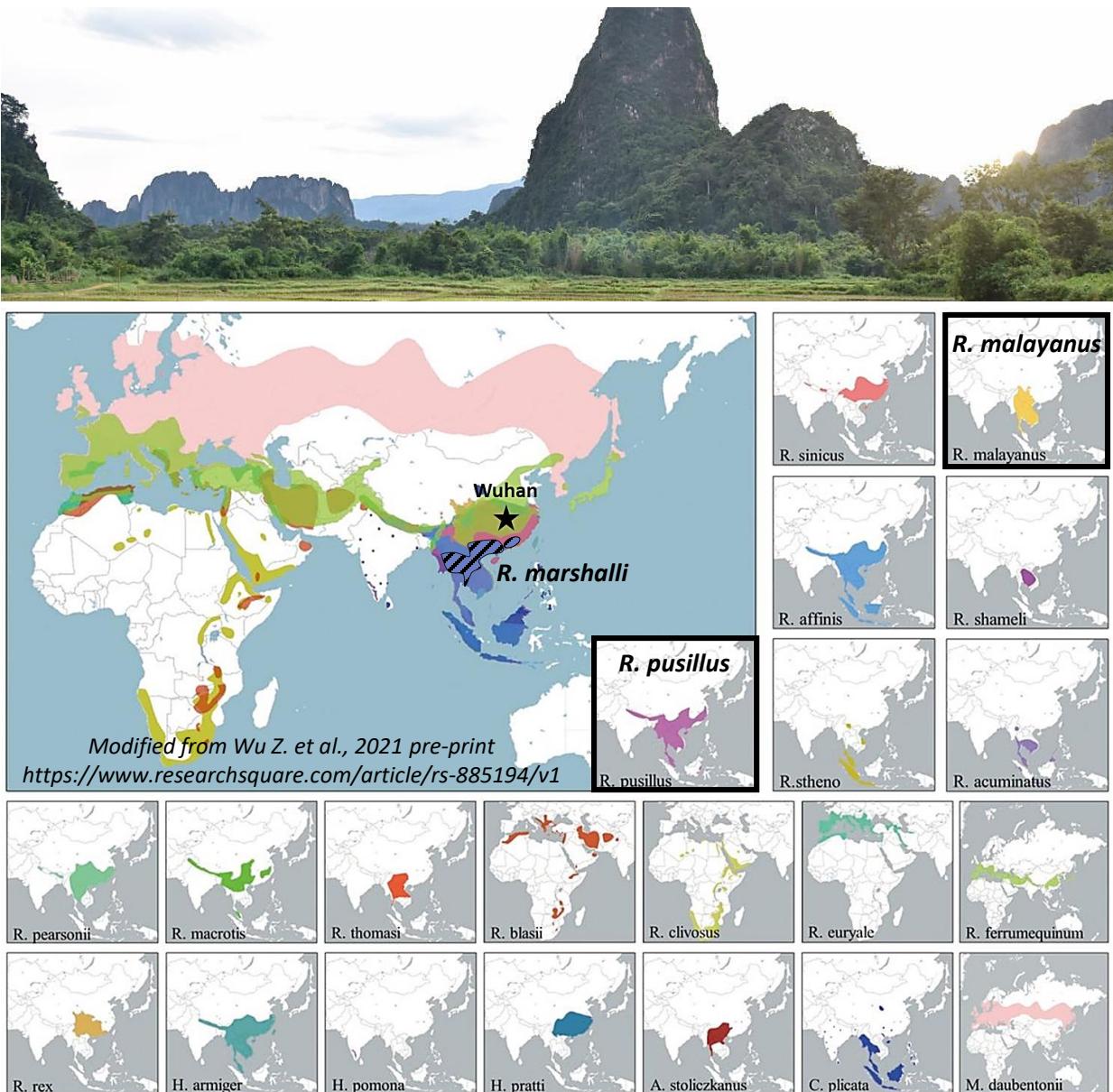
- Level of replication in classical or humanized mice
- Virus tropism in different organs
- Adaptation mutations
- Immune response to infection
- Cross-protection against SARS-CoV-2
- etc...

➤ Infection of Laotian populations?

- In 2020, low seroprevalence in humans
 - Impact of governmental measures?
 - Young age population?
or...
 - Cross-protective immunity?
- Serosurvey of general vs bat-exposed populations
 - LIPS: RBD, N, full S
 - PNT

Virachith et al., 2021 Lancet Reg Health West Pac.

Conclusions



➤ SARS-CoV-2 origin

- **Recombination** from different *Rhinolophus* CoVs living in the limestone cave system of S-E. Asia
 - Rich diversity of bats living in sympatry → ↗ exchanges of viruses
 - Major route of transmission: feces (but oral swabs also >0 for BANAL-27)
- **No need for an intermediate host** to increase affinity for ACE2, but...
 - Origin of the furin cleavage site?
- **Route of introduction** to the human population?

➤ Risk of emergence of other bat CoVs?

- **Exposure** of guano collectors, religious communities, tourists?
- If yes, **symptomatic** infection? Cross-protection against SARS-CoV-2?

Acknowledgments



Pathogen Discovery Laboratory

Sarah Temmam, Béatrice Regnault, Delphine Chrétien,
Souand Mohamed Ali, Nicolas Da Rocha, Philippe Pérot,
Thomas Bigot, Marc Eloit.

Molecular Genetics of RNA Viruses Unit

Sandie Munier, Flora Donati, Faustine Amara, Océane
Dehan, Angela Brisebarre, Mikaël Attia, Sylvie van der
Werf.

Structural Virology Unit

Eduard Baquero-Salazar, Félix A. Rey.

Structural Bioinformatics Unit

Massimiliano Bonomi, Yasaman Karami, Michael Nilges.

Mouse Genetics Unit

Laurine Conquet, Xavier Montagutelli.

- Biomics platform – IPP
- Covid taskforce – IPP
- LabEx IBEID – IPP

Financial support



ສະຖາບັນ ປັດສະເຕີ ລາວ
INSTITUT PASTEUR DU LAOS

Institut Pasteur du Laos

Khamsing Vongphayloth, Vincent Lacoste, Somphavanh
Somlor, Khaithong Lakeomany, Thonglakhone
Xaybounou, Somsanith Chonephetsarath, Nothasin
Phommavanh, Anthony Black, Paul T. Brey.



Faculty of Environmental Sciences

National University of Laos

Bounsavane Douangboubpha, Daosavanh Sanamxay,
Vilakhan Xayaphet, Phetphoumin Paphaphanh.

- H2020 ReCoVER – EU
- UK embassy grant
- Luxembourg Development special grant