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

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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, Somphavanh 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,+}

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* co-first authors

+ corresponding author

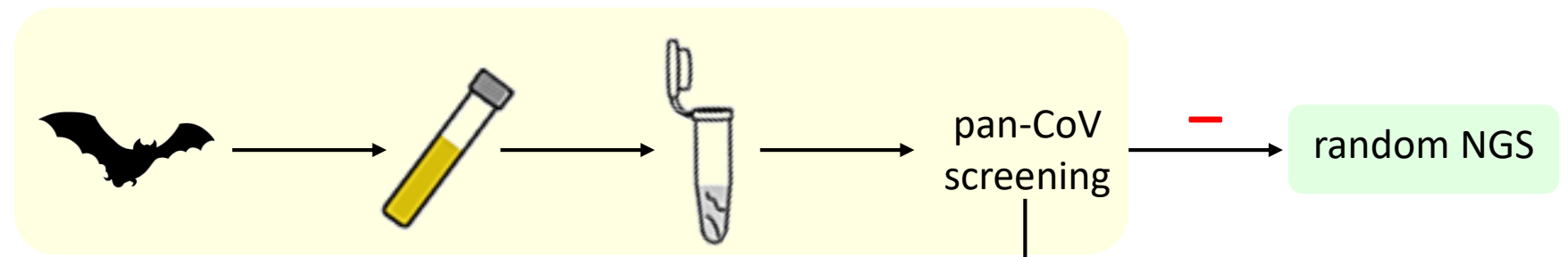
Objectives

- 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

- IP-Laos
- IPP-Pathogen discovery
- IPP-Structural virology
- IPP-Structural bioinformatics
- IPP-Molecular Genetics of RNA Viruses
- IPP-Mouse genetics



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



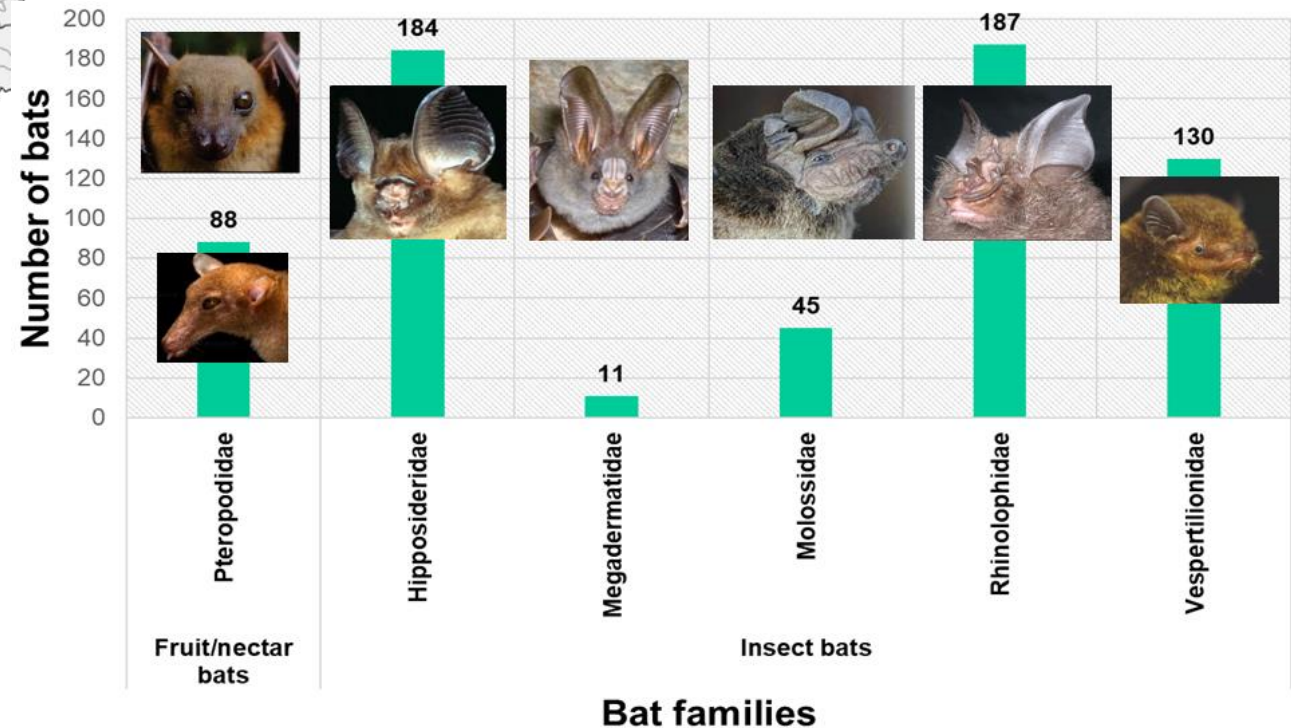
Harp trap



Mist net

- **645 bats**
 - 48 species
 - 6 families

- **Sample types**
 - Oral swabs (608)
 - Rectal swabs (539)
 - Urine (157)
 - Blood (246)



pan-CoV screening



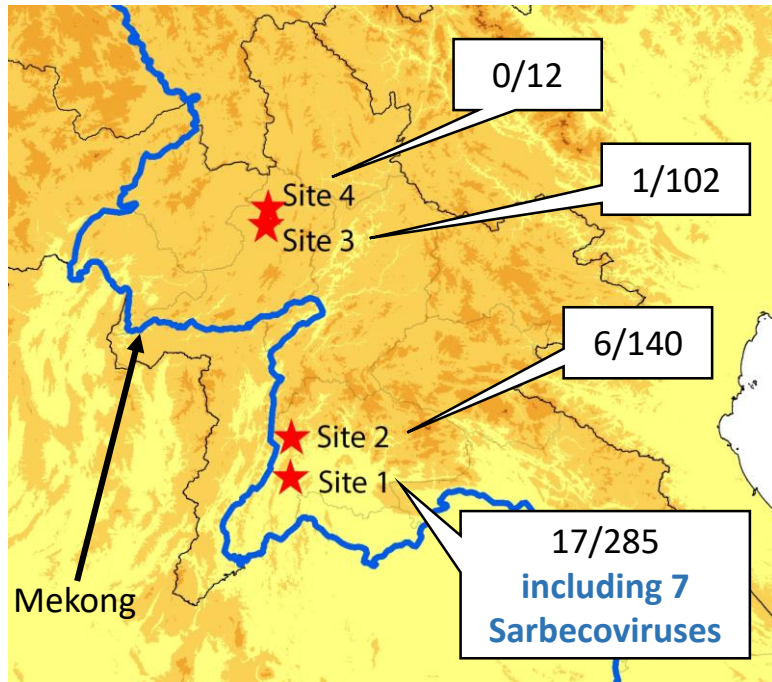
Vincent Lacoste
IP-Laos
(former member)

JOURNAL OF VIROLOGY, Dec. 2011, p. 12815–12820
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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%

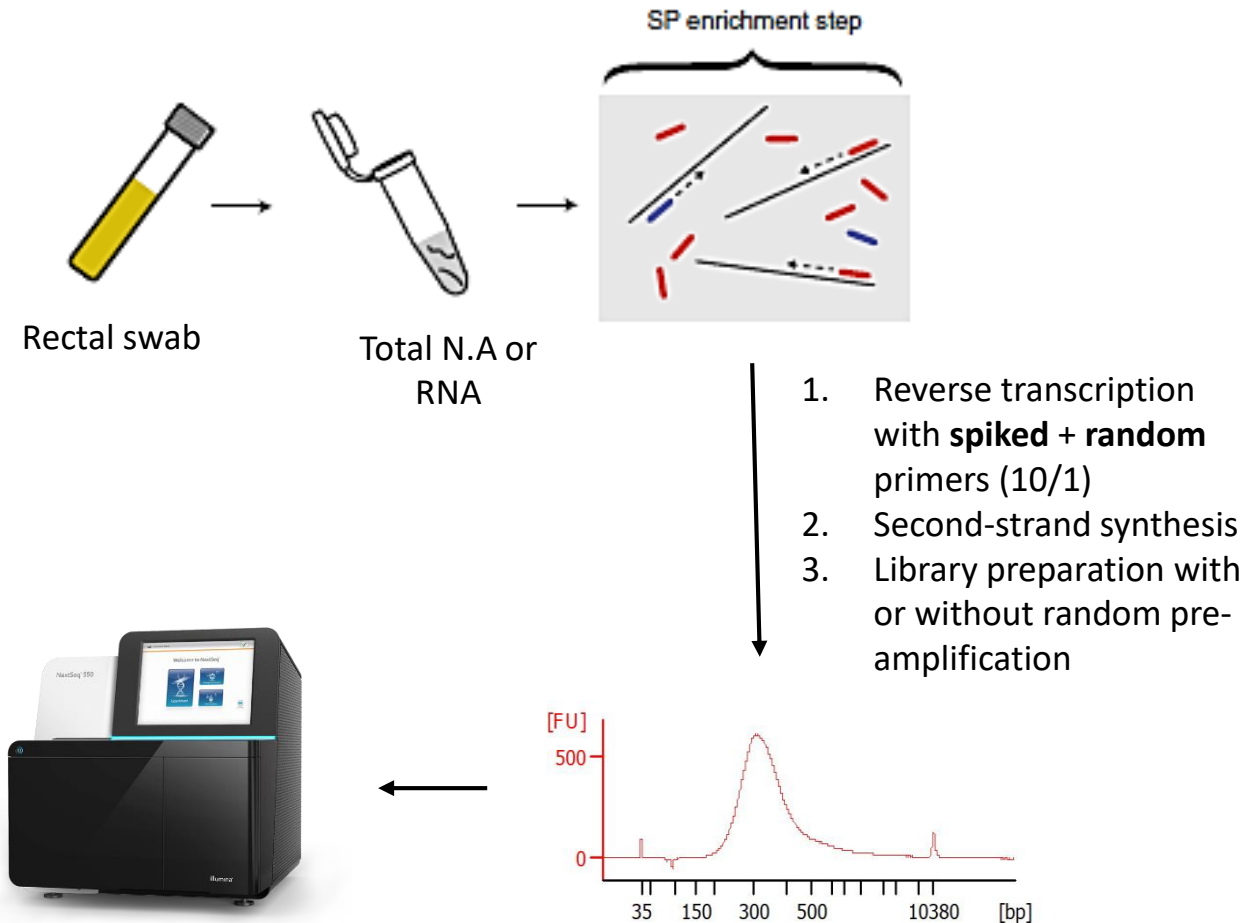
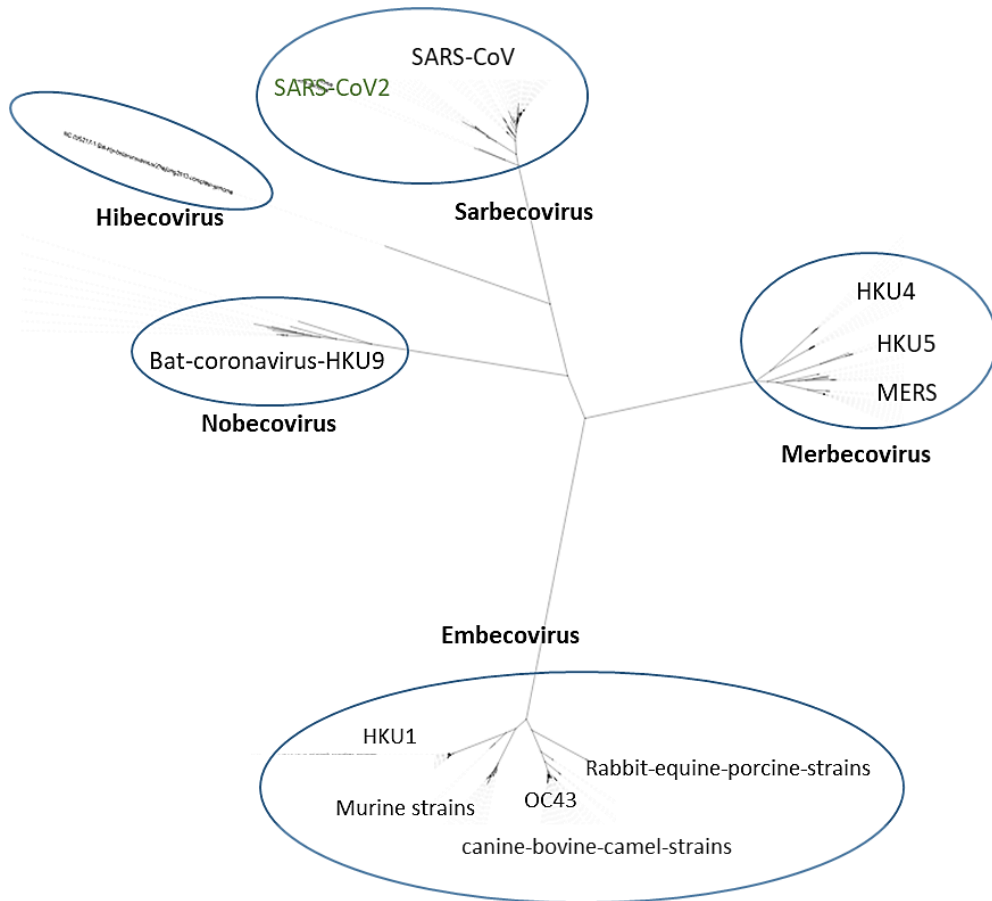
Betacoronavirus enrichment



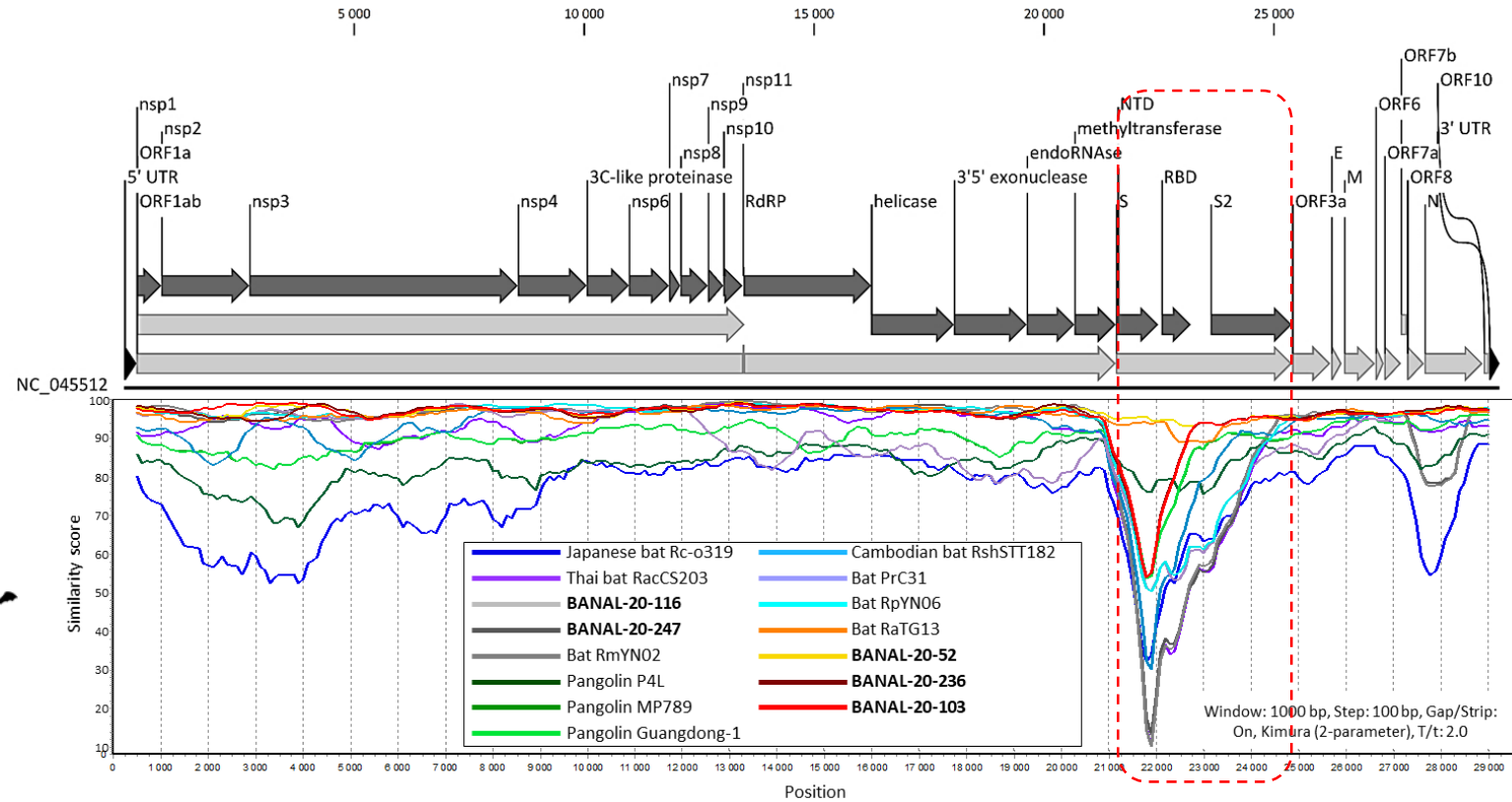
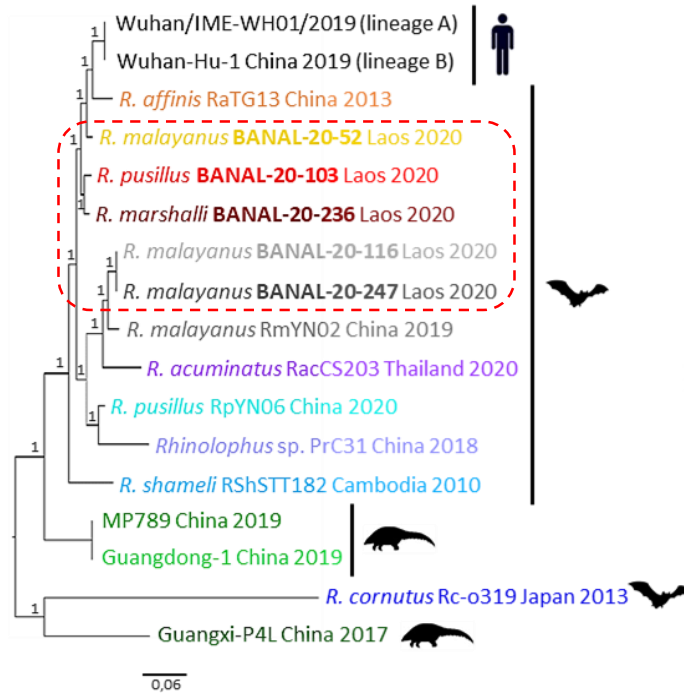
Béatrice Regnault
IPP – Pathogen
Discovery lab.

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

- 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



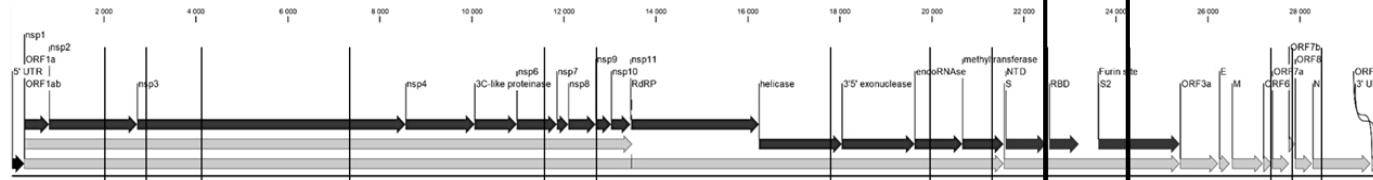
➤ At the whole genome level

- 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

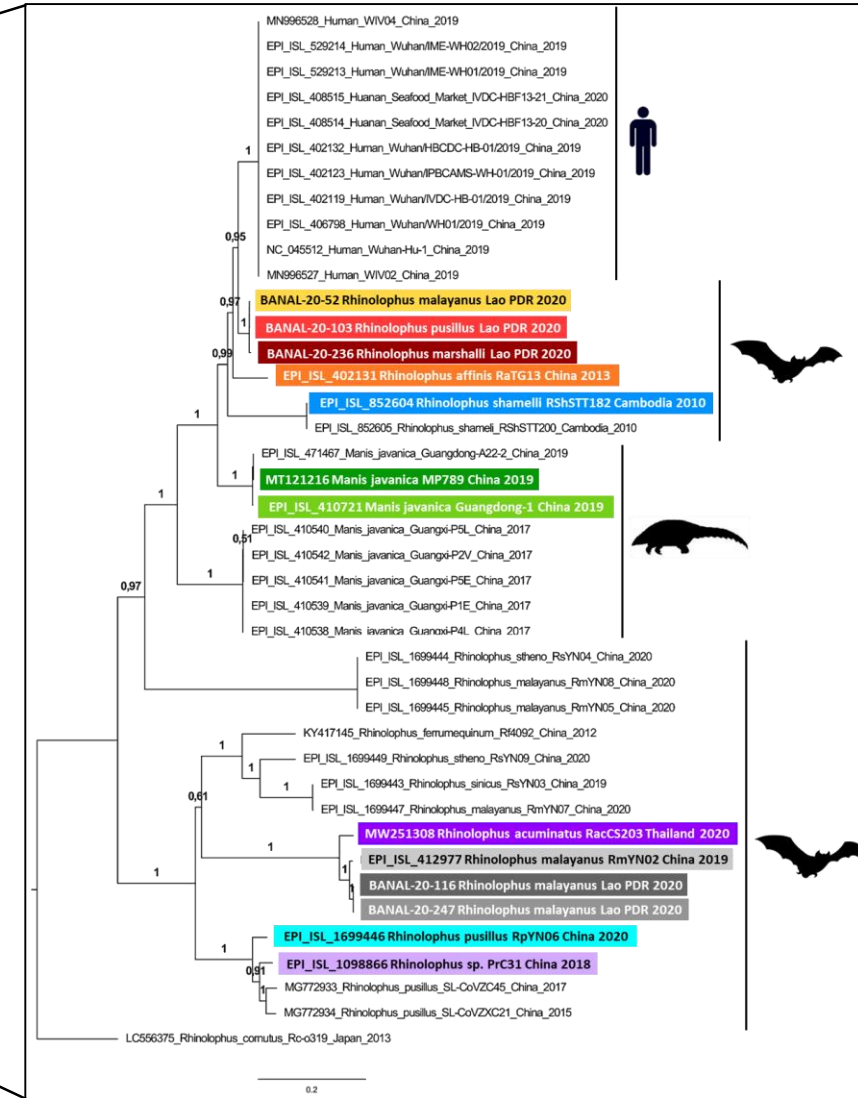


Fragment no:

Fragment no:	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
SARS-CoV-2	RmYN02	BANAL 103	MULT	MULT	RpYN06	MULT	MULT	RmYN02	BANAL 52	RaTG13	MULT	MULT	BANAL 52	MULT	
Bat RaTG13	MULT	MULT	MULT	MULT	MULT	MULT	MULT	MULT	MULT	SARS CoV-2	MULT	MULT	MULT	MULT	
BANAL-20-52	BANAL-236		BANAL-103		MULT		BANAL-236		MULT	MULT	MULT	MULT		SARS CoV-2	MULT
BANAL-20-103	MULT	MULT	BANAL-52		MULT	MULT	MULT	MULT	MULT	BANAL-236		MULT	MULT	MULT	MULT
BANAL-20-236	BANAL-52	RacCS 203	MULT		BANAL-247		BANAL-52		MULT	BANAL-103		MULT	MULT	MULT	MULT
Pangolin Guangdong-1							MP789				MULT		MP789		MP789
Pangolin MP789							Guangdong-1				Guangdong A22-2		Guangdong-1		Guangdong-1
Bat PrC31	MULT		RmYN02		MULT	MULT	Rf4092		MULT	RpYN06		MULT		MULT	RpYN06
Bat RpYN06	MULT	MULT	MULT	MULT		SARS-CoV-2		RmYN02		MULT	BANAL 236	PrC31	MULT	RmYN02	PrC31
Cambodian bat RShSTT182															RShSTT200
BANAL-20-116			BANAL-247			MULT				BANAL-247					BANAL-247
BANAL-20-247			BANAL-116		BANAL-236					BANAL-116					BANAL-116
Bat RmYN02	SARS-CoV-2		PrC31		MULT		RpYN06		SARS-CoV-2	MULT	MULT	MULT	RpYN06	MULT	MULT
Thai bat RacCS203	MULT	MULT	BANAL 236		MULT	MULT	MULT	MULT	MULT	MULT	MULT	MULT	MULT	MULT	MULT

➤ 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)

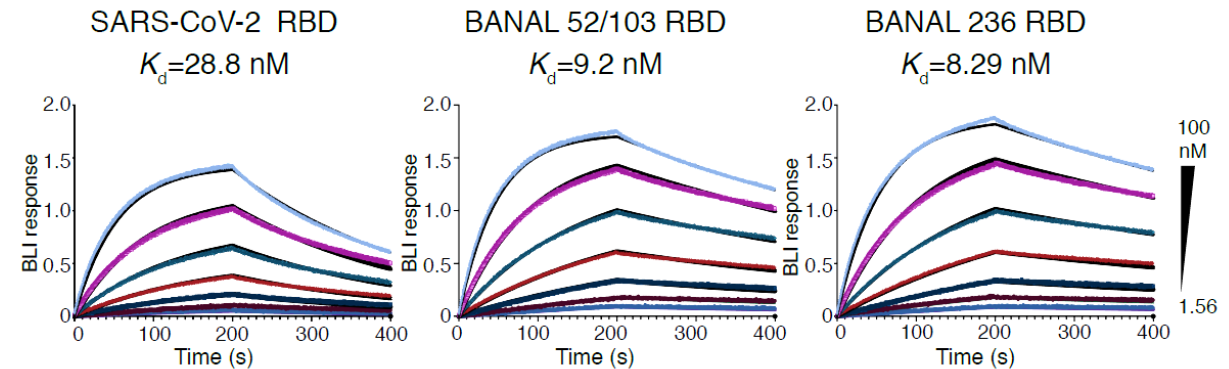
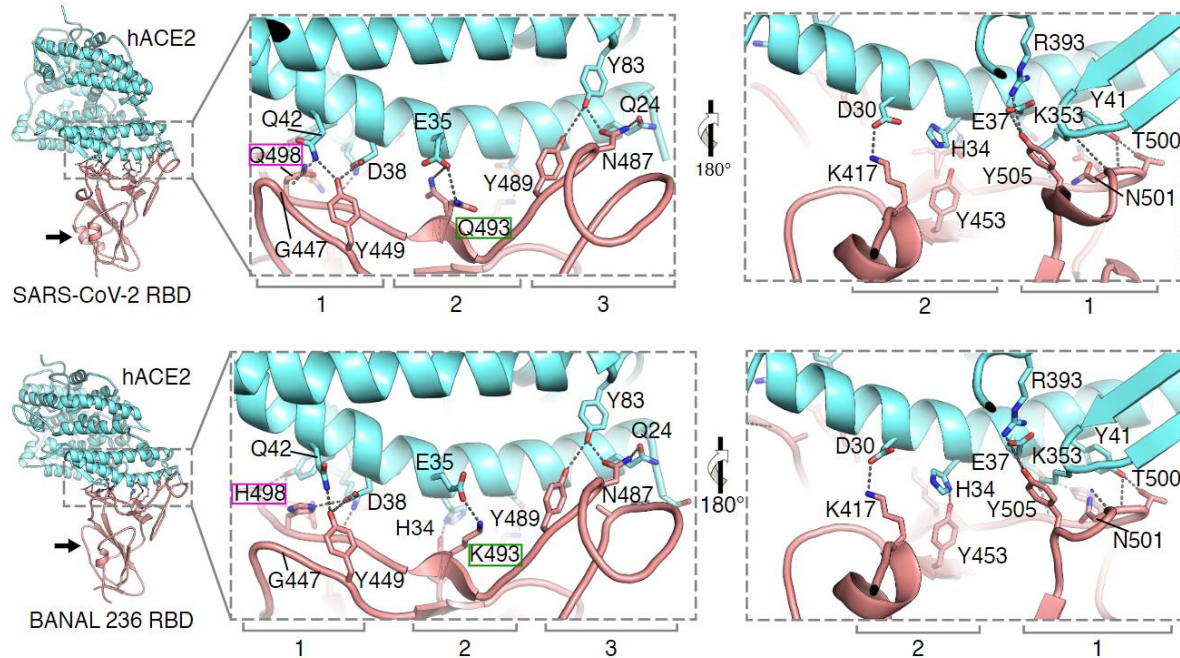


Insights into RBD/hACE2 complex



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 $H498^Q$ disrupt transient hydrogen bonds but is known to increase affinity of SARS-CoV-2 RBD for hACE2 *Huang et al., 2021 EBioMedicine*
- BANAL mutations $K493^Q \rightarrow$ 2 additional salt bridges formed between BANAL-236 RBD & ACE2 \rightarrow possible \nearrow affinity

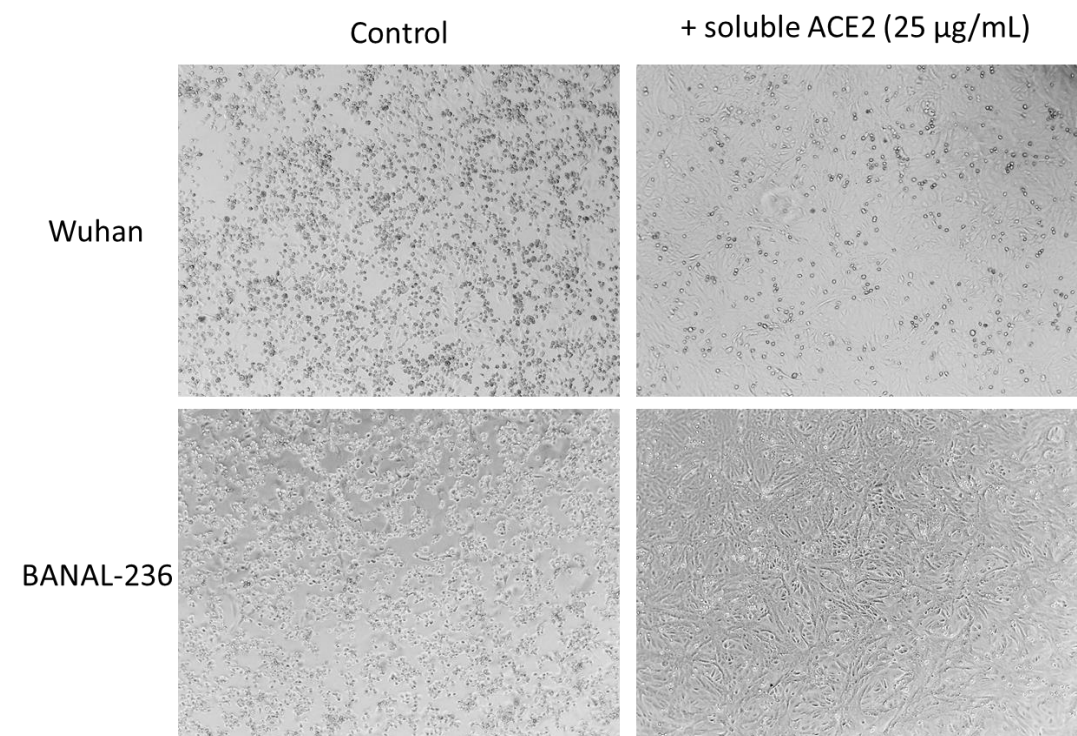
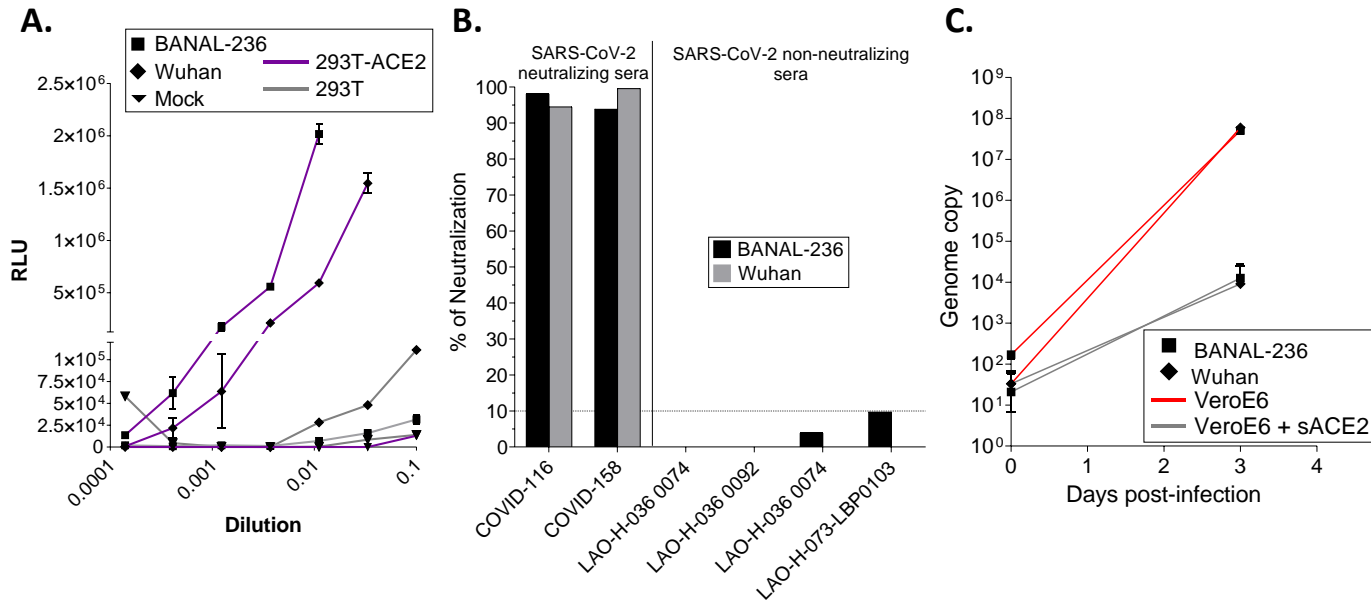
➤ Biolayer interferometry

- K_d BANAL viruses \ll SARS-CoV-2 \rightarrow \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 log₁₀) similar to Wuhan (-3,8 log₁₀)

Virus isolation

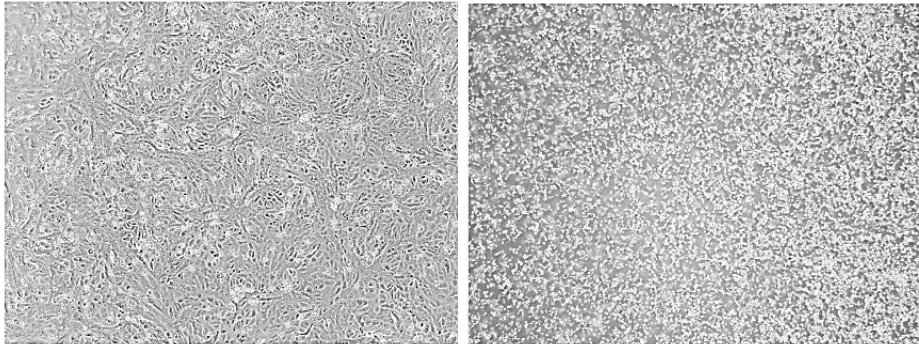


Flora Donati
IPP – Molecular
Genetics of RNA
Viruses

Neg control

BANAL-236

VeroE6

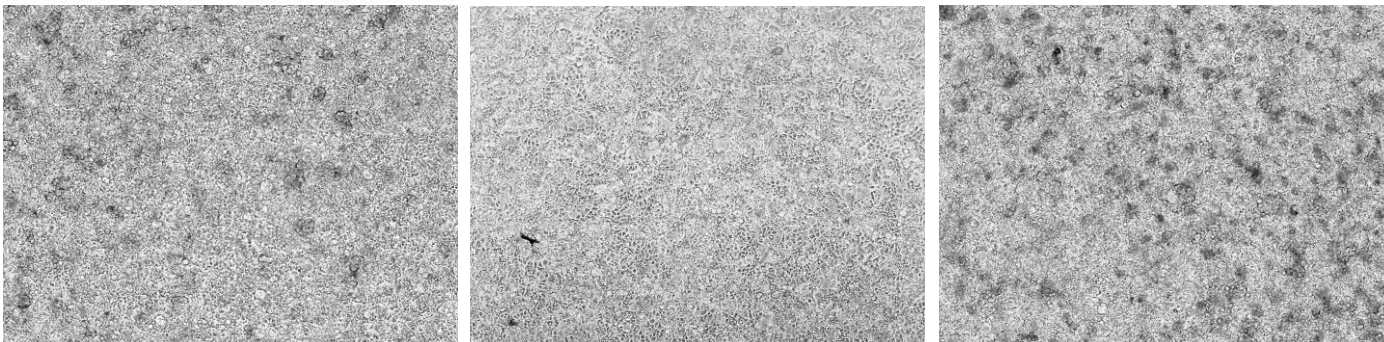


➤ BANAL-236 virus growth

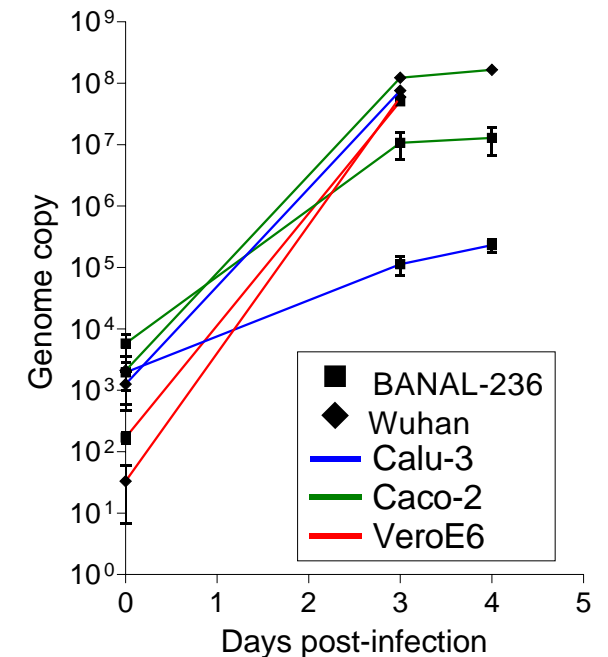
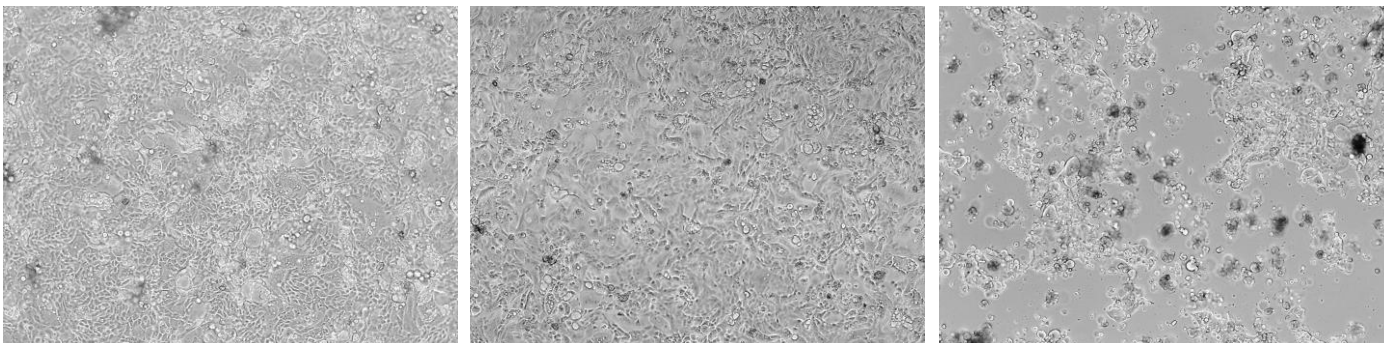
- Efficient replication in VeroE6, similar yield compared to Wuhan
- Lower replication in human cells expressing endogenous ACE2
 - No CPE observed compared to Wuhan

Wuhan

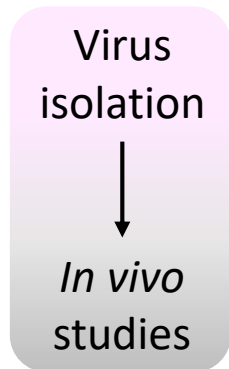
Caco-2



Calu-3



Ongoing studies

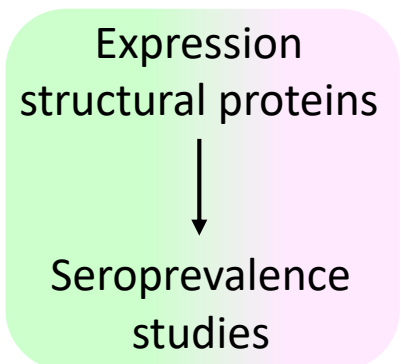


➤ Pathogenicity in animal model (mice + primates)

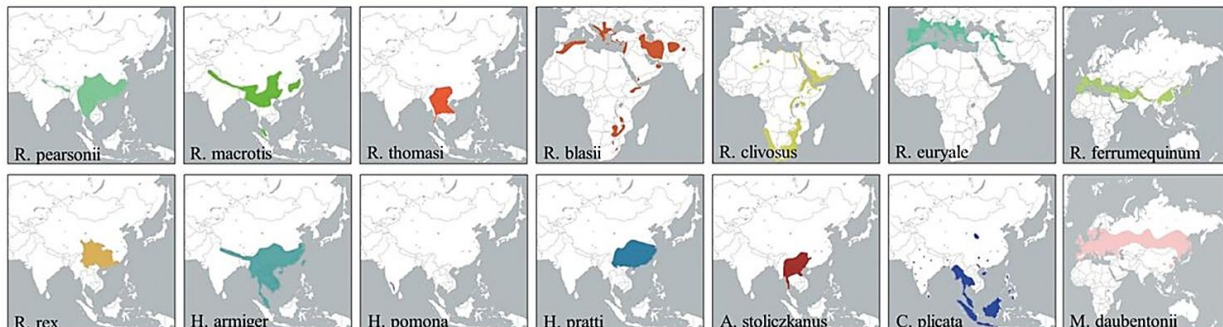
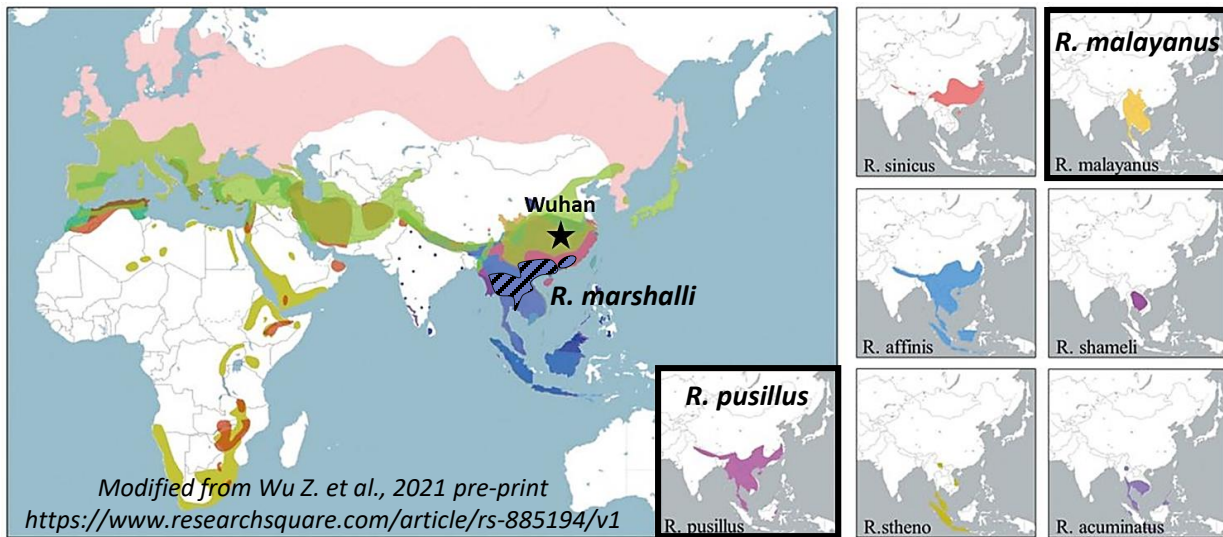
- Level of replication in classical or humanized mice
- Virus tropism in different organs
- Adaptation mutations
- Immune response to infection
- Cross-protection against SARS-CoV-2
- etc...

➤ Infection of Laotian populations?

- In 2020, low seroprevalence in humans Virachith et al., 2021 Lancet Reg Health West Pac.
 - Impact of governmental measures?
 - Young age population?
or...
 - Cross-protective immunity?
- Serosurvey of general vs bat-exposed populations
 - LIPS: RBD, N, full S
 - PNT



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

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Structural Bioinformatics Unit

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Mouse Genetics Unit

Laurine Conquet, Xavier Montagutelli.



ສະຖາບັນ ປັດສະເຕີ ລາວ
INSTITUT PASTEUR DU LAOS

Institut Pasteur du Laos

Khamsing Vongphayloth, Vincent Lacoste, Somphavanh Somlor, Khaithong Lakeomany, Thonglakhone Xaybounsou, 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.

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