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ARTEMISININ RESISTANCE IN *PLASMODIUM FALCIPARUM* IN MALARIA ENDEMIC AREAS, LAO PDR

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Background:

- Artemisinin-based combination therapies (ACTs) are the first-line antimalarial treatments comprising a short-acting artemisinin drug for rapid parasitemia reduction plus a long-acting partner drug to eliminate surviving parasites that can cause recrudescences.
- Artemisinin can clear the bulk of infection rapidly, but small numbers of persistent parasites must be removed by the partner drug.
- **Artemisinin resistance:** a delay in the clearance of malaria parasites from the bloodstream following treatment with an ACT.

  ⇨ less effective in clearing all parasites within a 3-day period

ACTs: Artemisinin-based combination therapies

Background:

- Despite the fact that malaria morbidity and mortality have decreased in Laos, artemisinin-resistant *P.f* has been reported since 2013, which is now threatening malaria elimination in the country by 2030.

- **Objective**: to assess the distribution of the k13-mutations in Laos, which are reported to be responsible gene mutations for artemisinin-resistance of the parasite

P. falciparum =Plasmodium falciparum

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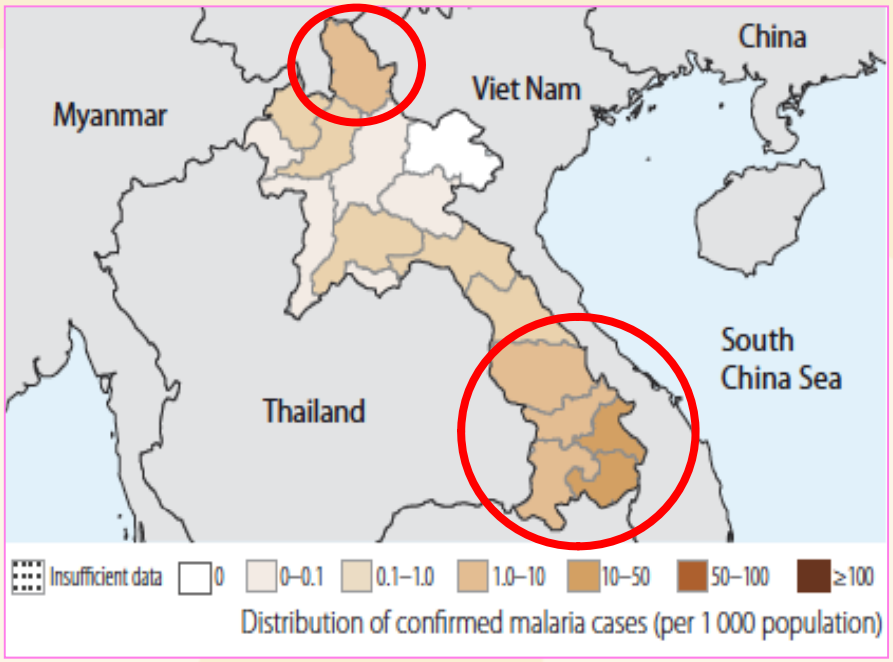
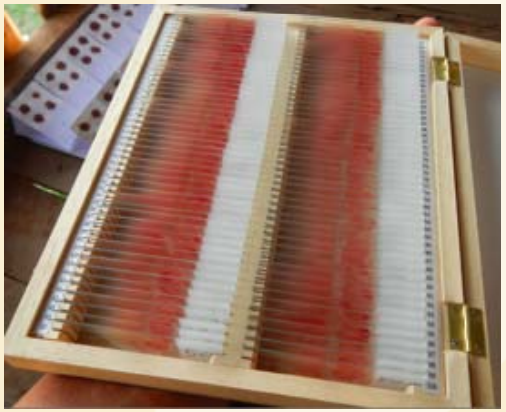
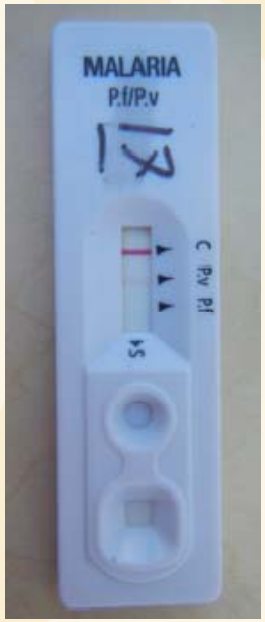
Method:

- Blood samples of malaria patients were collected in 160 health care facilities in 5 southern provinces from October 2015 to November 2016, where over 90% of malaria cases in Laos were reported.
- The blood collection was also conducted in the northern-most province, Phongsaly, from November-December 2017.
- Real-time PCR was performed to identify *Plasmodium* species and direct DNA sequencing was performed to detect the k13 mutations.

Blood samples collection in 5 southern provinces and the northern-most province







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Blood samples collection in
5 southern provinces and the northern-most province

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Results:

- In the 5 southern provinces, a total of 1,553 cases of *P. falciparum* were detected, in which, 52.8% had nonsynonymous mutations in the k13-mutations, precisely,
  - Attapeu (107 cases which occupied 76.9% of the samples);
  - Champasak (n=384, 67.7%);
  - Sekong (n=72, 48.3%);
  - Salavan (n=150, 53.2%);
  - Savannakhet (n=107, 24.9%).
- The C580Y was the most predominant mutation in the k13, followed by R539T, Y493H and P574L.
- In Phongsaly (Northern-most province adjacent to China): 8 malaria cases including 3 *P. falciparum*, 3 *P. malariae* and 2 *P. vivax*.
  - All the 3 *P. falciparum* possessed the C580Y mutation.

P. falciparum(Pf) and *Pf* + *P. vivax(Pv)*

	Oct. 2015-Apr. 2016			May 2016-Nov. 2016		
	Pf	***Pf+Pv***	**total**	***Pf***	***Pf+Pv***	**total**
Savannakhet	246	8	254	142	24	166
Salavan	200	16	216	48	2	50
Sekong	112	28	140	16	15	31
Chanpasack	384	34	418	82	47	129
Attapeu	114	9	123	25	1	26
Total	1,056	95	1,151	313	89	402

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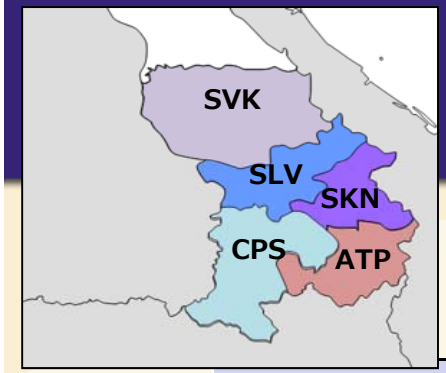

Results of K13 gene analysis
K13 artemisinin resistant mutations
Sampling period: Oct. 2015-Apr. 2016

North

South

Area	Nonsynonymous mutation	Wild type	Total
SVK	71 (28.0%)	183 (72.0%)	254
SLV	126 (58.3%)	90 (41.7%)	216
SKN	54 (38.6%)	86 (61.4%)	140
CPS	303 (72.5%)	115 (27.5%)	418
ATP	85 (69.1%)	38 (30.9%)	123

Average Mutation Rate; 55.5%

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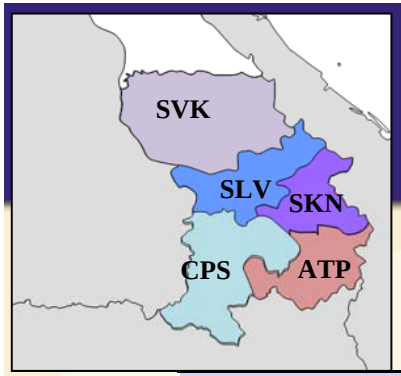

Results of K13 gene analysis
K13 artemisinin resistant mutations
Sampling period: May-Nov. 2016

North

South

Area	Nonsynonymous mutation	Wild type	Total
SVK	36 (21.7%)	130 (78.3%)	166
SLV	24 (48.0%)	26 (52.0%)	50
SKN	18 (58.0%)	13 (42.0%)	31
CPS	81 (62.8%)	48 (37.2%)	129
ATP	22 (84.6%)	4 (15.4%)	26

Average Mutation Rate; 45.0%

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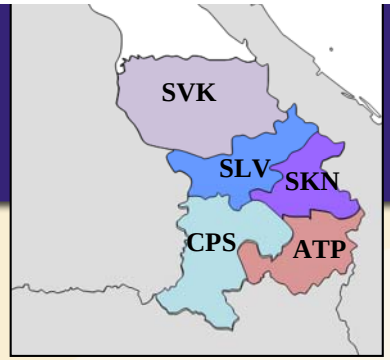
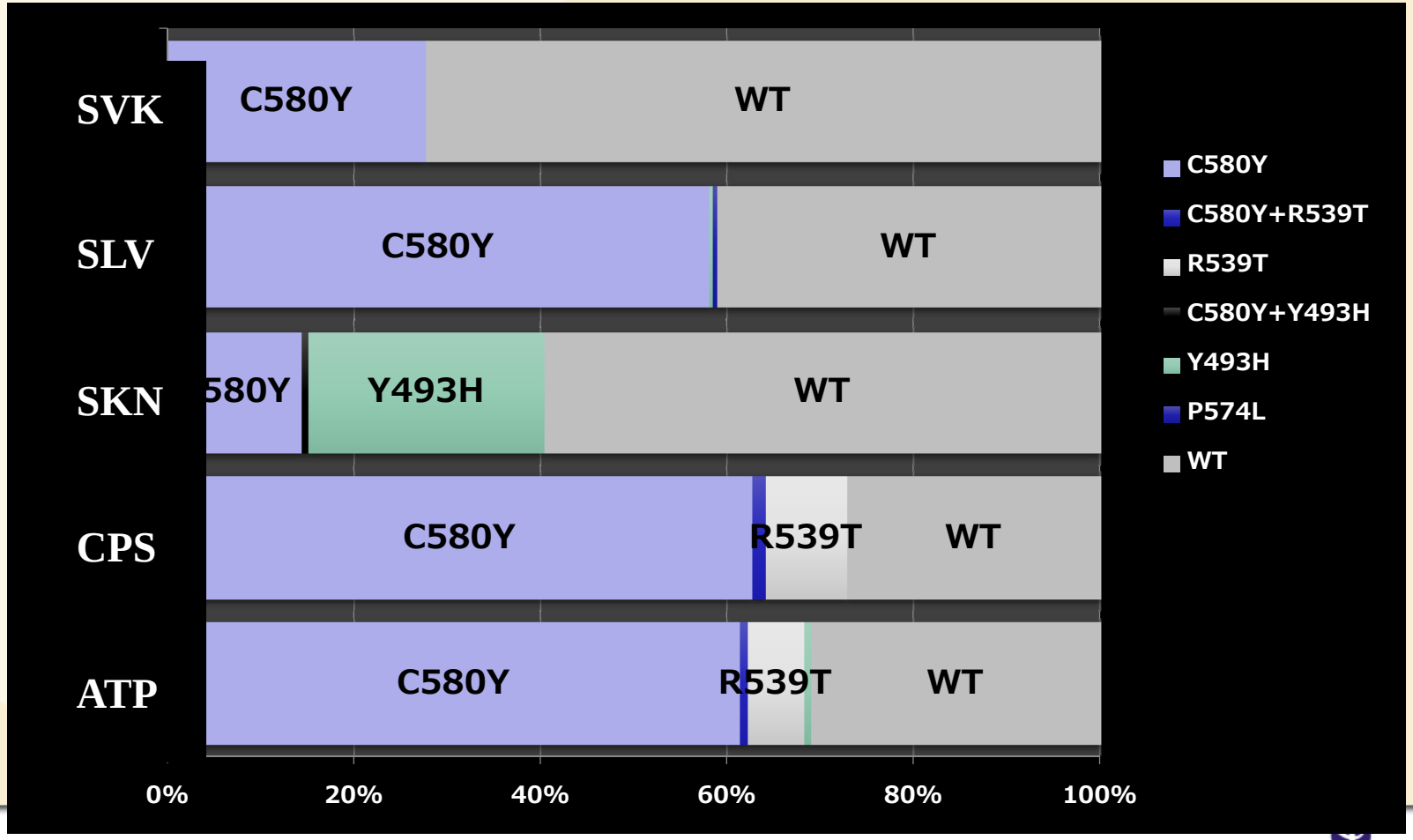


Results of K13 gene analysis
Types of the resistant mutations
Sampling period: Oct. 2015-Apr. 2016

North

South



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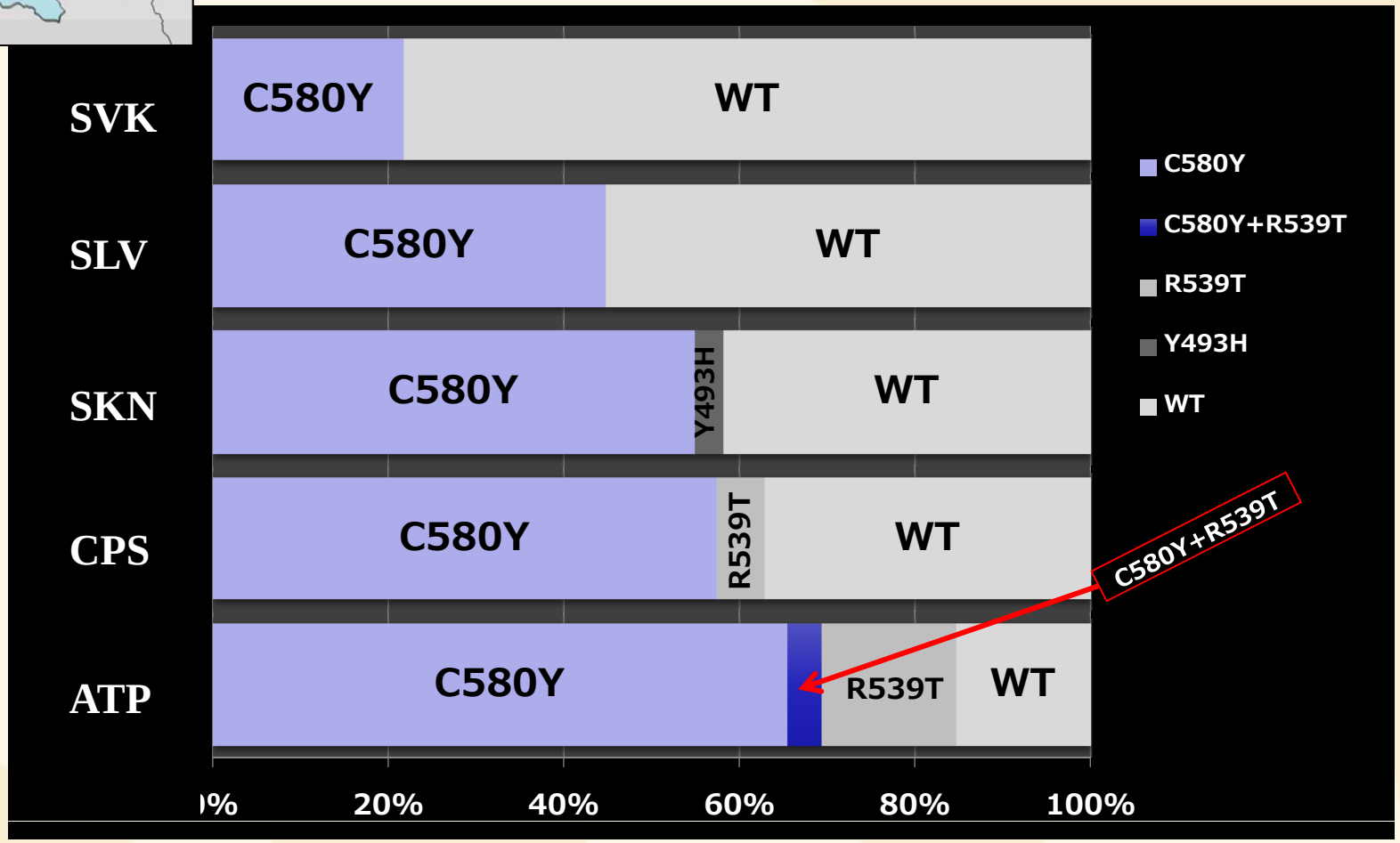
Results of K13 gene analysis
Types of the resistant mutations
Sampling period: May-Nov. 2016

North

South



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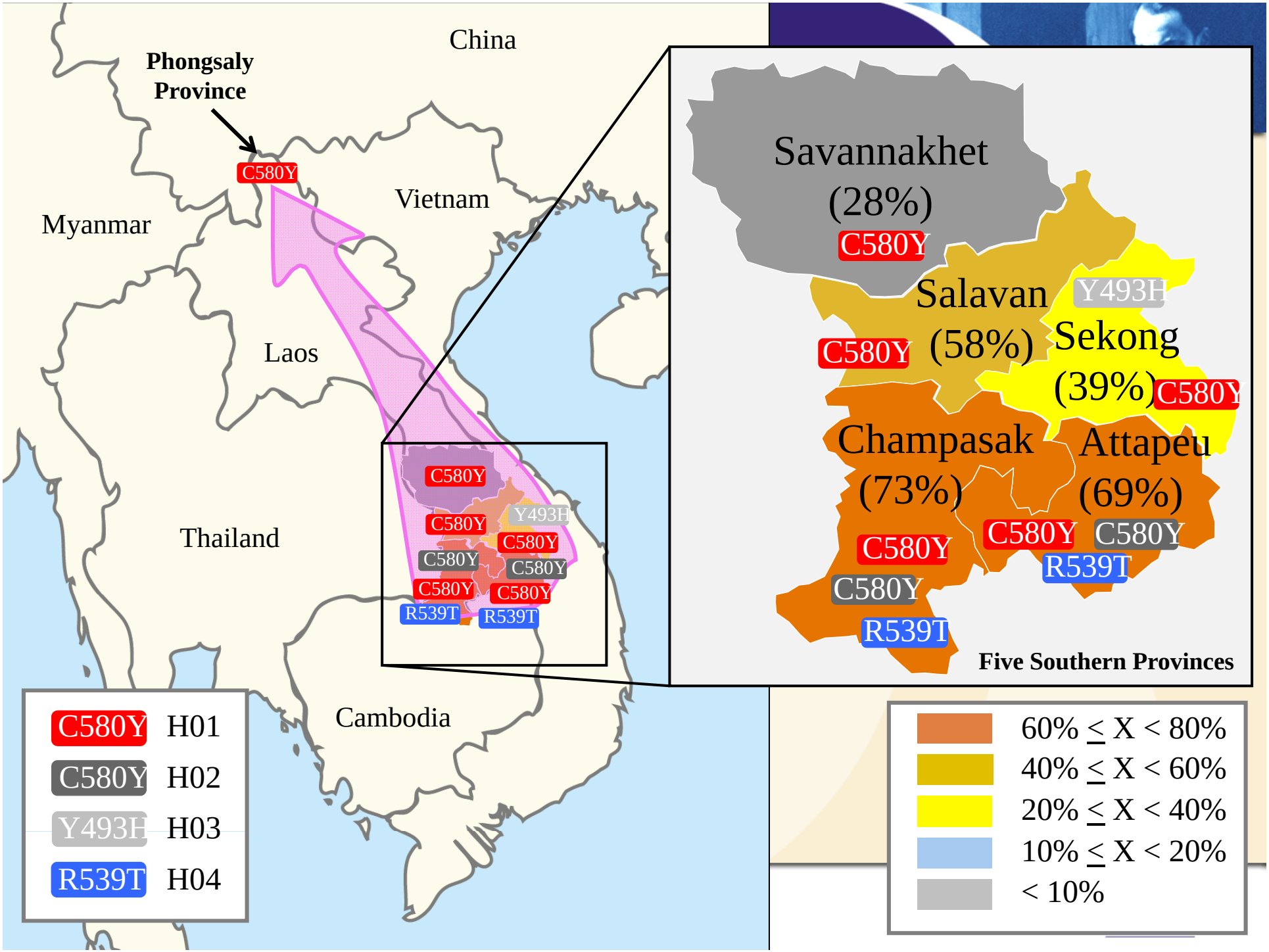
China
Phongsaly Province
C580Y
Vietnam
Myanmar
Laos
Thailand
Cambodia
C580Y
Y493H
C580Y
C580Y
C580Y
C580Y
C580Y
C580Y
R539T
R539T
Savannakhet (28%)
C580Y
Salavan (58%)
C580Y
Y493H
Sekong (39%)
C580Y
Champasak (73%)
C580Y
C580Y
Attapeu (69%)
C580Y
C580Y
R539T
R539T
Five Southern Provinces
C580Y H01
C580Y H02
Y493H H03
R539T H04
60% ≤ X < 80%
40% ≤ X < 60%
20% ≤ X < 40%
10% ≤ X < 20%
< 10%

Conclusion:

- The C580Y was the predominant mutation among the 4 mutations in these 6 provinces.
- Artemisinin-resistant *P. falciparum* has already reached to the Chinese boarder of Laos
- The high rate of the k13-mutations highlights the importance of surveillance of artemisinin-resistance to achieve the elimination of malaria in Laos, as well as in the Greater Mekong Sub-region.

Thank you for your attention

SATREPS

Science and Technology Research Partnership for Sustainable Development Program

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