

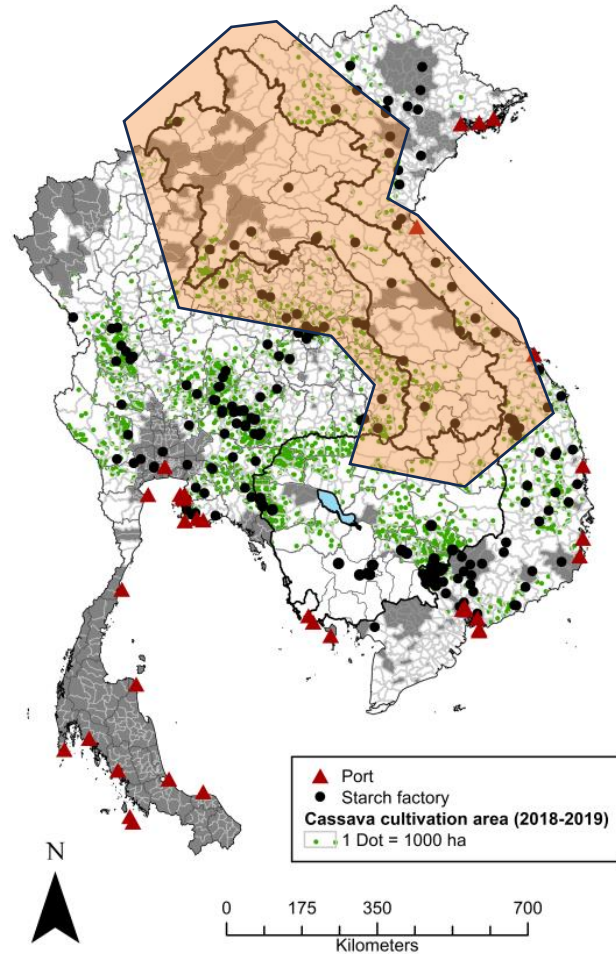


Cassava disease management: Knowing the insect vector *Bemisia tabaci* in Laos

Ana María Leiva, Khonesavanh Chittarath, Diana López-Alvarez, Pinkham Vongphachanh, **María Isabel Gómez**, Somkhith Sengsay, Xiao-Wei Wang, Rafael Rodríguez, Jonathan Newby and Wilmer J. Cuellar

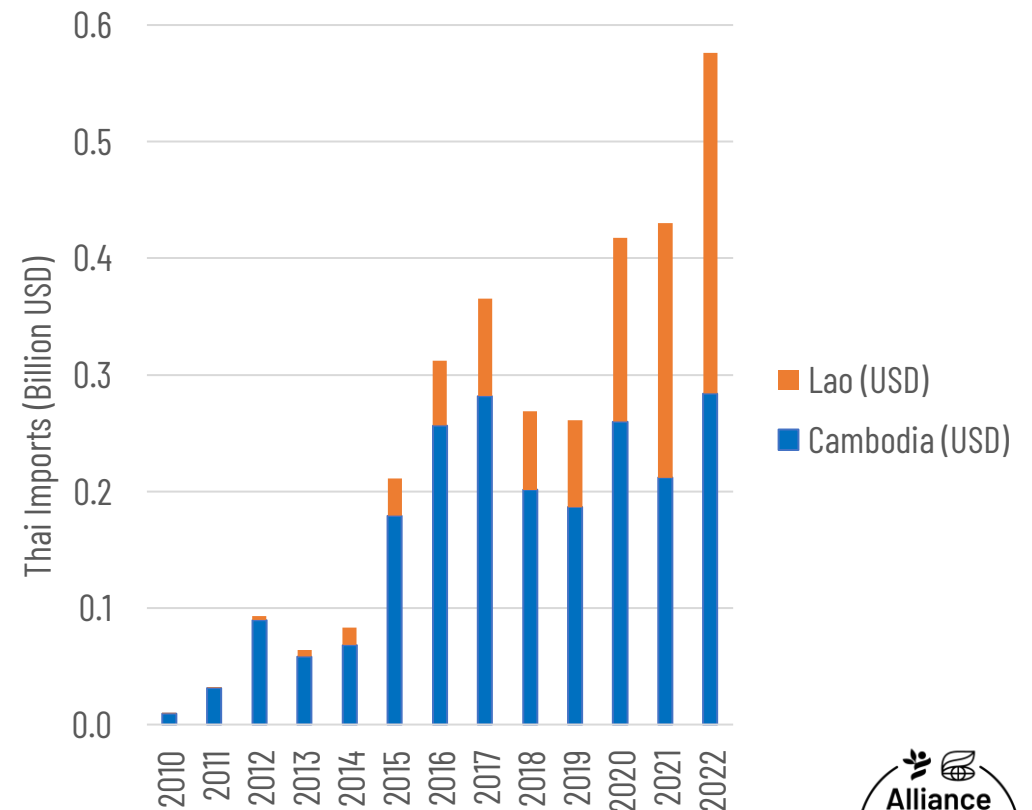
Palmira, Colombia
June 27th, 2023

South East Asia (SEA) – increased cassava production



~ 3.3 Billion USD exports for Thailand
~ 1.4 Billion USD exports for Vietnam
~ 1.2 Billion USD imports from Cambodia
~0.35 Billion USD export from Lao PDR

Cassava has become an important crop in terms of both rural livelihoods and economic development in Southeast Asia. In Laos, has been traditionally grown as a subsistence crop, but in recent years cassava cultivation in this country has expanded and is becoming a “cash crop” for farmers (Malik et al., 2020).



Cassava Mosaic Disease



Cassava Mosaic Disease in the world

Built with [pestdisplace/Geminivirus](#). Maintained by [PestDisPlace](#).

Showing 739 of 739 genomes sampled between Dec 1986 and Jun 2020.

Virus ^

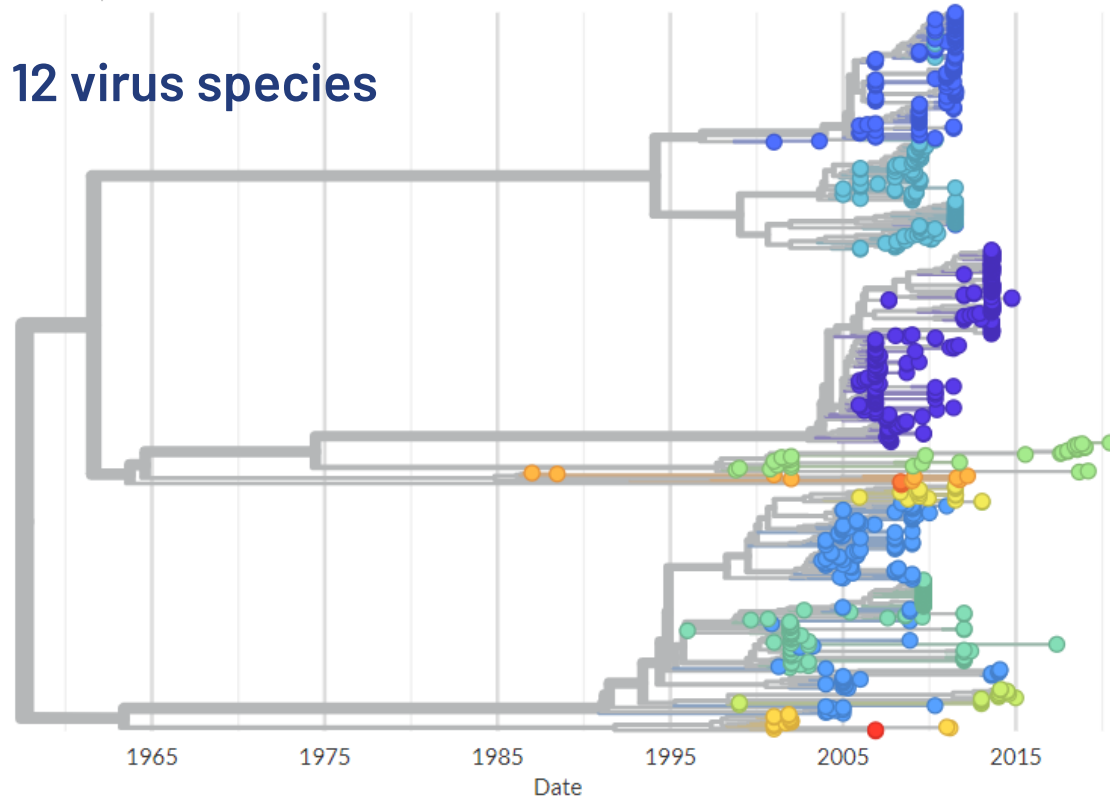
- ACMV
- SACMV
- EACMV
- EACMKV
- EACMV UG
- SLCMV
- EACMMV
- EACMCV
- EACMZV
- ICMV
- ACMBFV
- CMMGV

Phylogeny

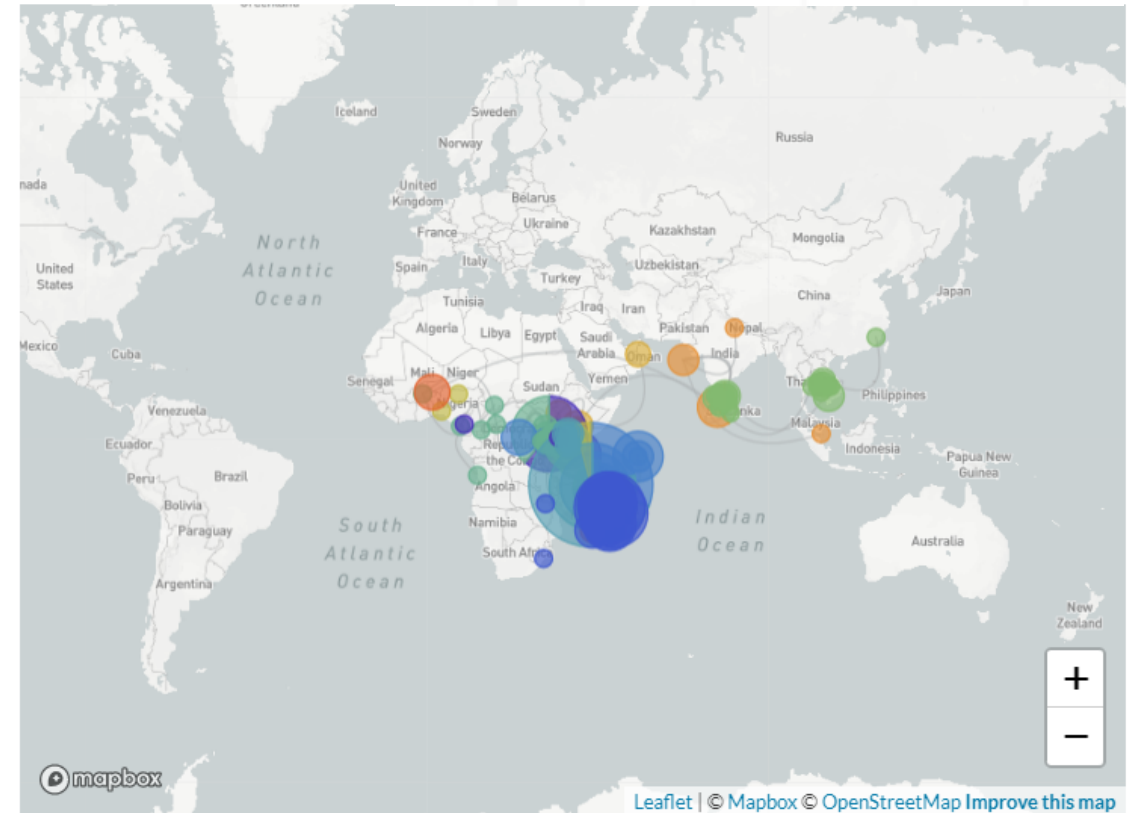
Virus v

ZOOM TO SELECTED RESET LAYOUT

12 virus species



Transmissions



Sri Lanka Cassava Mosaic Virus (SLCMV) was **first reported in Cambodia in 2015** and is now present throughout the major producing regions adding constraints to farmers already dealing with climate and market uncertainty.

Draw on global experience to develop a regional control plan September 2018

The overall project aim is to enhance smallholder livelihoods and economic development in mainland SEA by improving the resilience of cassava production systems and value chains by addressing the rapidly evolving disease constraints.

Establishing sustainable solutions to
cassava diseases in mainland Southeast
Asia



And why whiteflies are important when you are studying Geminivirus?

44 reproductively isolated but morphologically indistinguishable cryptic species of *Bemisia tabaci* complex



In the last years in Cambodia and Vietnam, the outbreaks of CMD caused by SLCMV were found to be associated with *Bemisia tabaci* Asia II 1 whiteflies, the only known efficient vector for SLCMV (Wang et al., 2016; Uke et al., 2018; Y. Chi et al., 2020)

Y. Chi, et al.

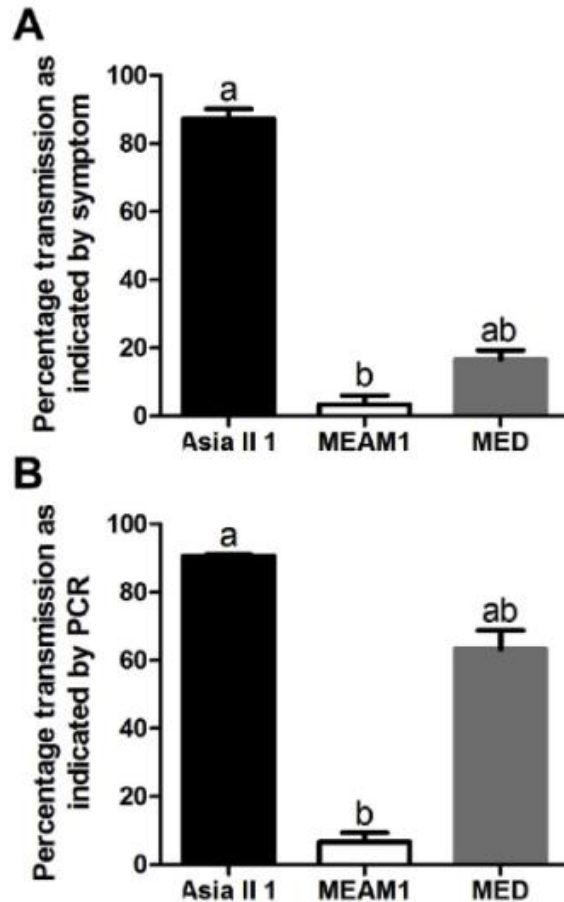


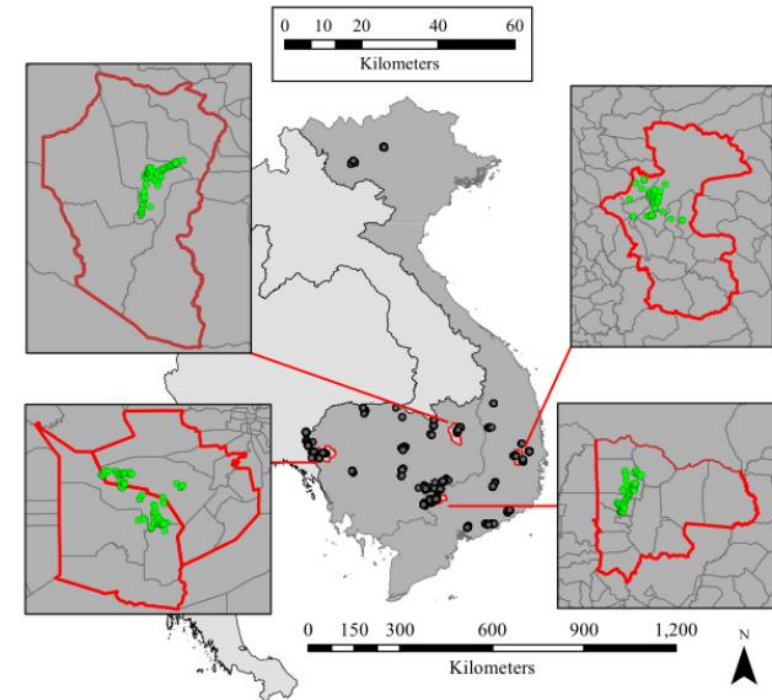
Fig. 1. Transmission efficiency of SLCMV to tobacco by three species of the *B. tabaci* complex (Asia II 1, MEAM1 and MED). Whiteflies were allowed a 96 h

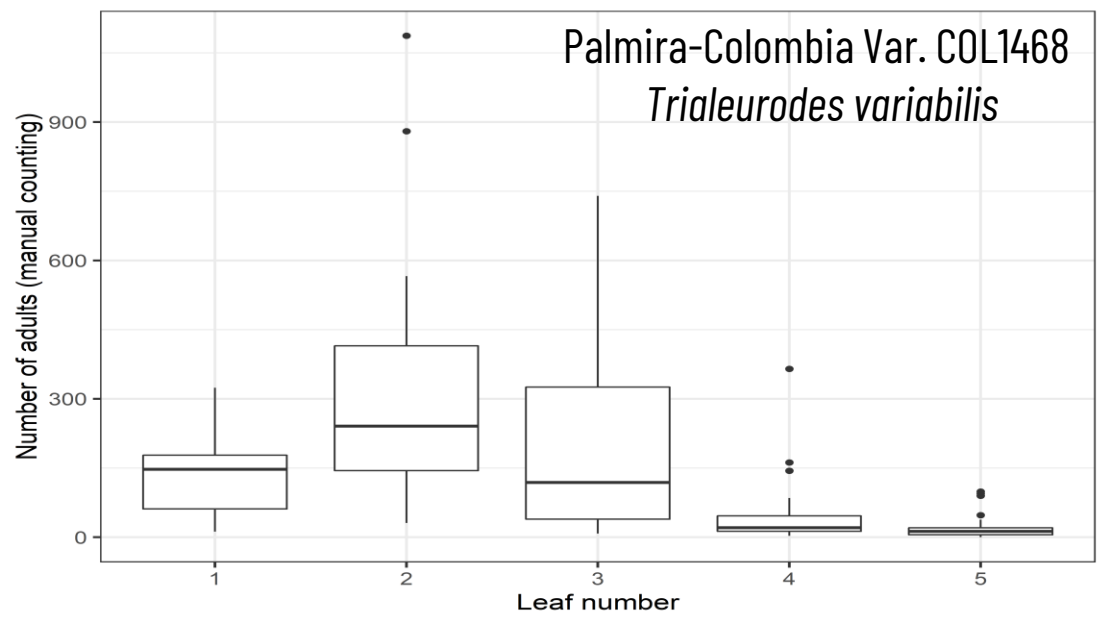
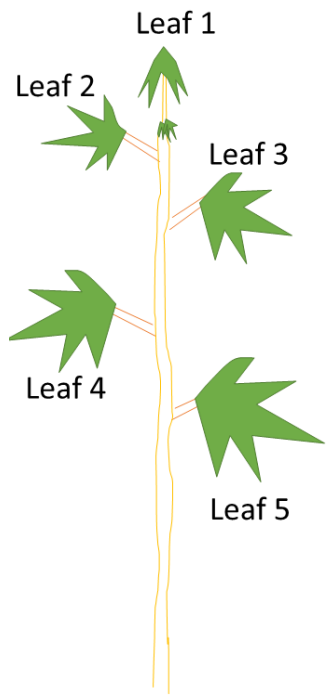


Annual report

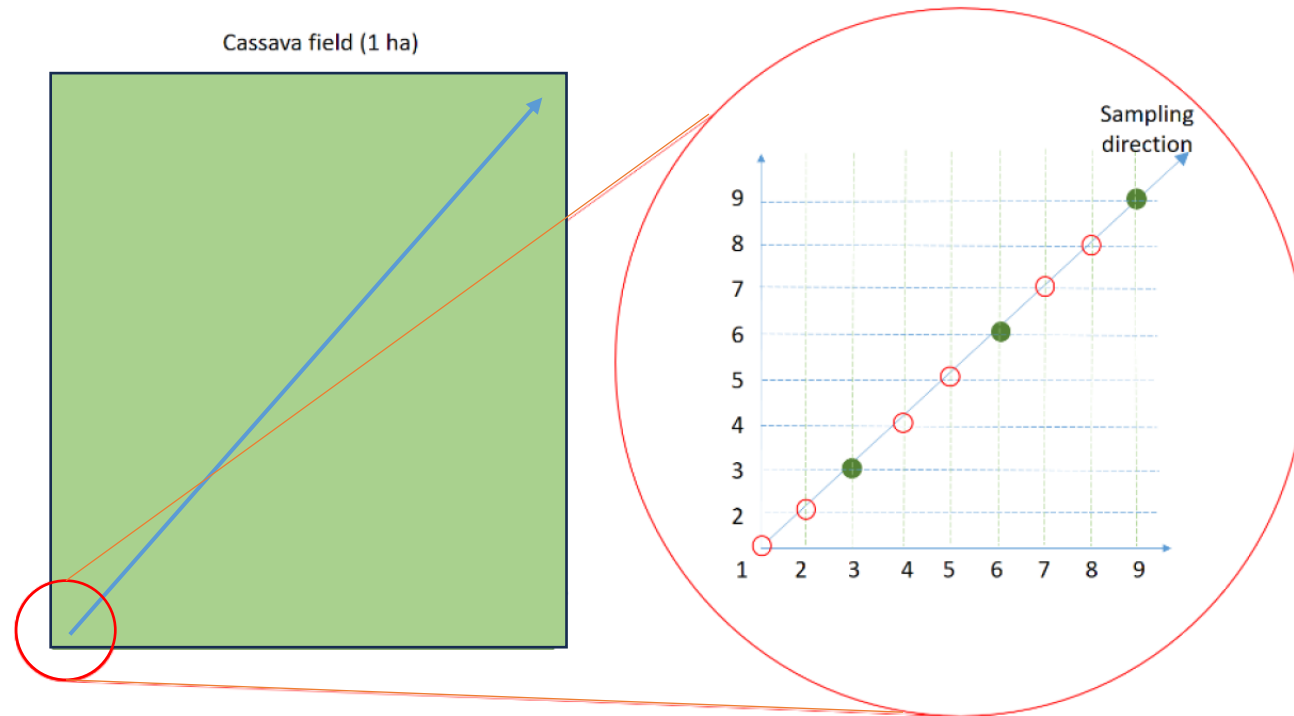
Short Research Activity: Developing an emergency response and long term management strategy for Cassava Mosaic Virus in Cambodia and Vietnam

In 2016 from 150 whitefly samples collected in Cambodia and Vietnam, 149 were identified as Asia II 1 and one as Asia II 6.

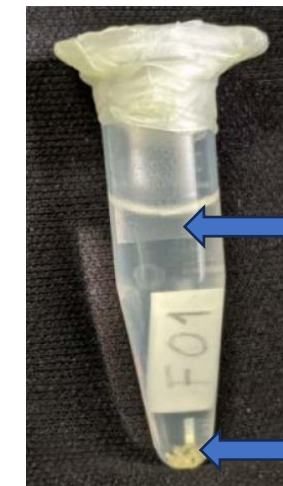
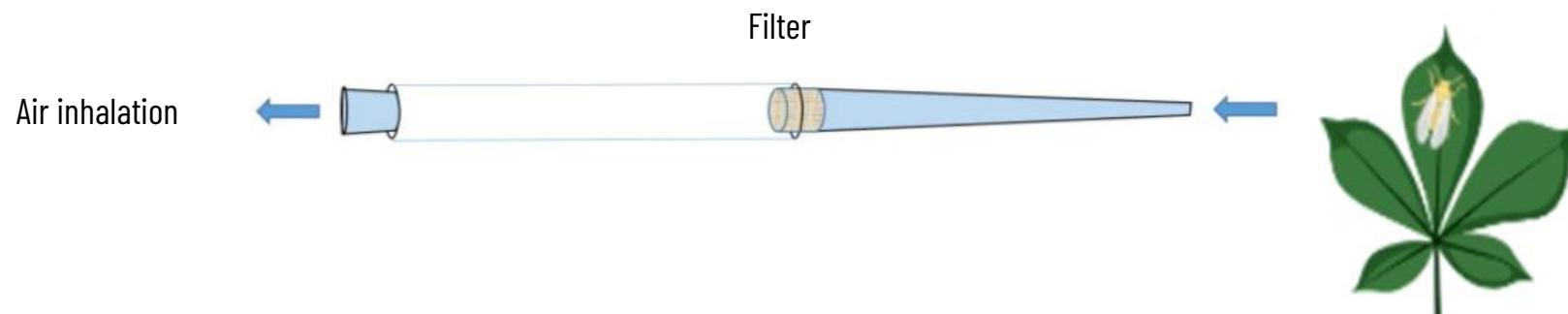




Abundance and diversity of whiteflies



“Starting at one corner of the field, take samples and the picture of the first expanded leaf, every 3rd plant following a diagonal transect (30 plants)”



Ethyl alcohol 96%

Whiteflies

Relative abundance of the potential vector

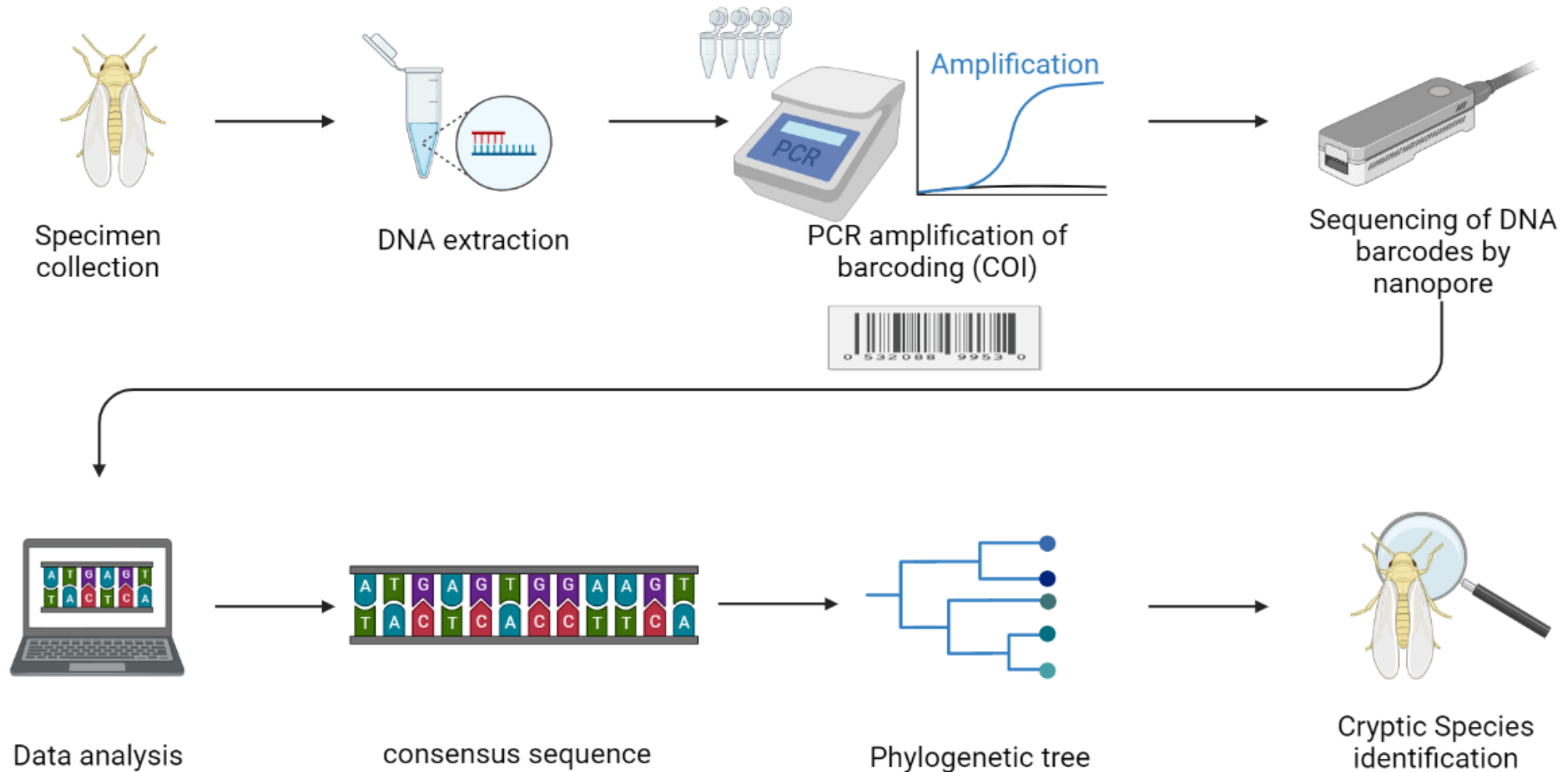
Table 1. List of surveyed provinces and abundance data for whiteflies associated to cassava in Lao PDR. Comparative whitefly abundance data from Tanzania and Nigeria is included. SD = standard deviation. The asterisks indicate provinces where CMD was observed. Asia II 1 and its haplotypes are indicated in red.

Country	Province	No. of Fields	Abundance (Per Leaf)	SD	Cryptic Species
Lao PDR	LaoungNamTha	2	0.150	0.012	Asia II 1
	Saiyabouly	9	0.041	0.021	Asia II 1
	Vientiane	5	0.014	0.012	Asia II 1/Asia II 6
	Xaysomboun	3	0.078	0.040	Asia II 1
	Borlikhamsai	11	0.145	0.034	Asia II 1/Asia II 6
	Khammoun	1	0.166	0.000	Asia II 1
	Salavan	7	0.129	0.031	Asia II 1/Asia II 6
	Sekong	1	0.167	0.000	Asia II 6
	Champasack *	25	0.177	0.043	Asia II 1/Asia II 6
Attapue *	6	0.233	0.072	Asia II 1/Asia II 6	
Tanzania			2.35–71.99 (14.3)	0.86–22.07	SSA1-SG1-SG2-SG3
Nigeria		24	2.34–265.5		SSA1-SG5, SSA3, SSA1-SG1-Bemisia afer, MED-ASL



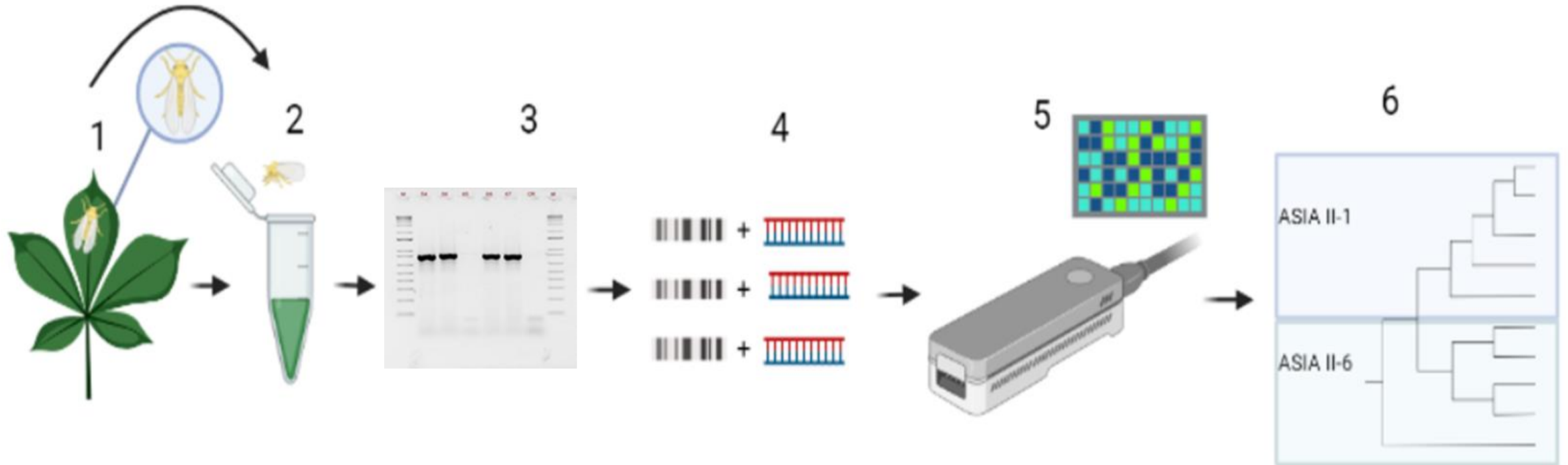
How could we know what cryptic species are in each locality, if they are not distinguishable morphologically?

DNA BARCODING FOR INSECT



DNA barcoding refers to the technique of sequencing a short fragment of the mitochondrial cytochrome c oxidase subunit I (COI) gene

Methodology used in the Laboratory



1—Collected sample, 2— Direct Polymerase Chain Reaction (PCR) using the whole whitefly, 3— Confirm the PCR by electrophoresis, 4—library preparation, 5— Sequencing using Nanopore Tech, 6— Data analysis.

Technology transfer



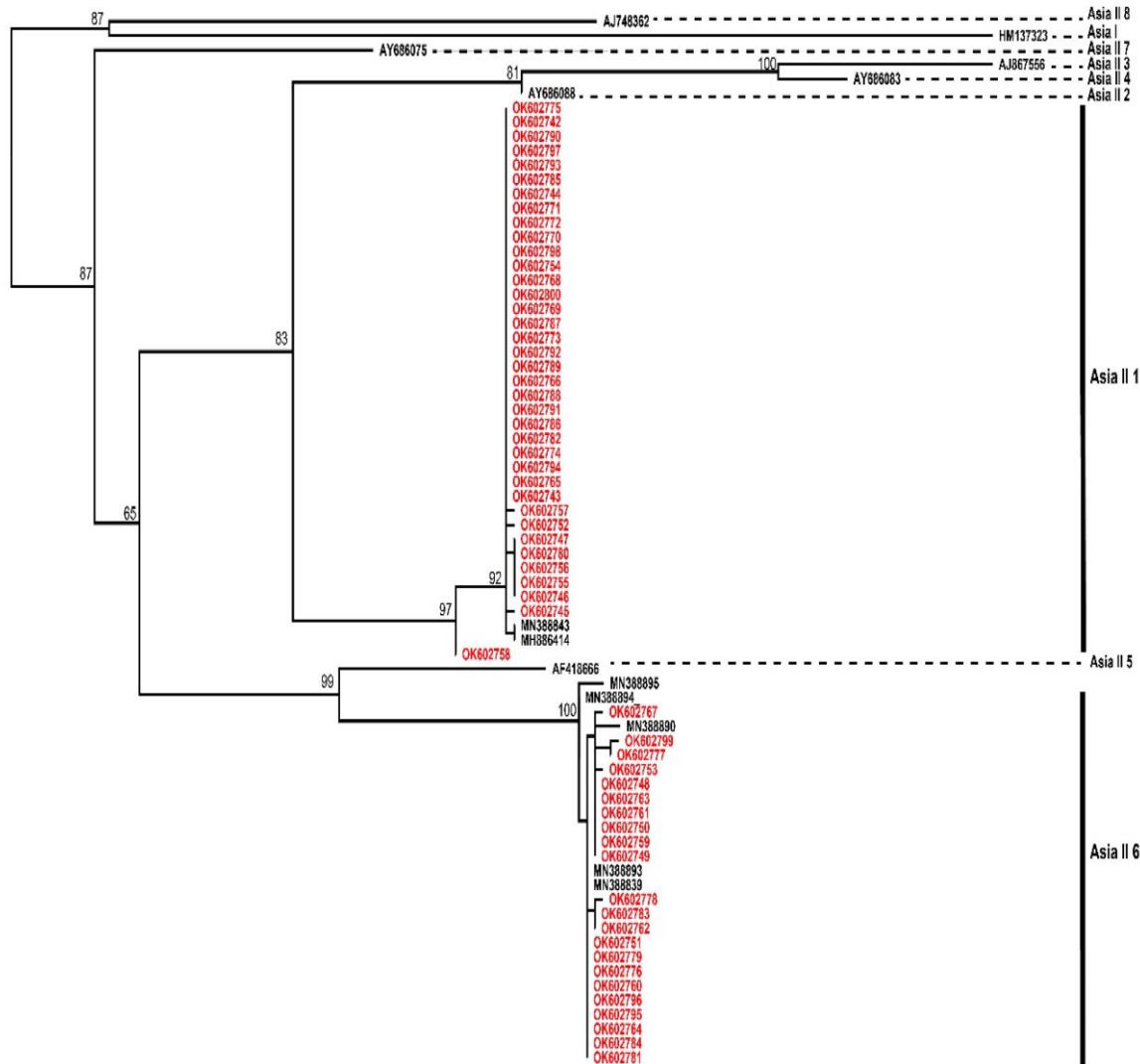
Oxford Nanopores Course in Laos:

- Introduction to sequencing technology
- Develop human capacities in the region



RESULT

A

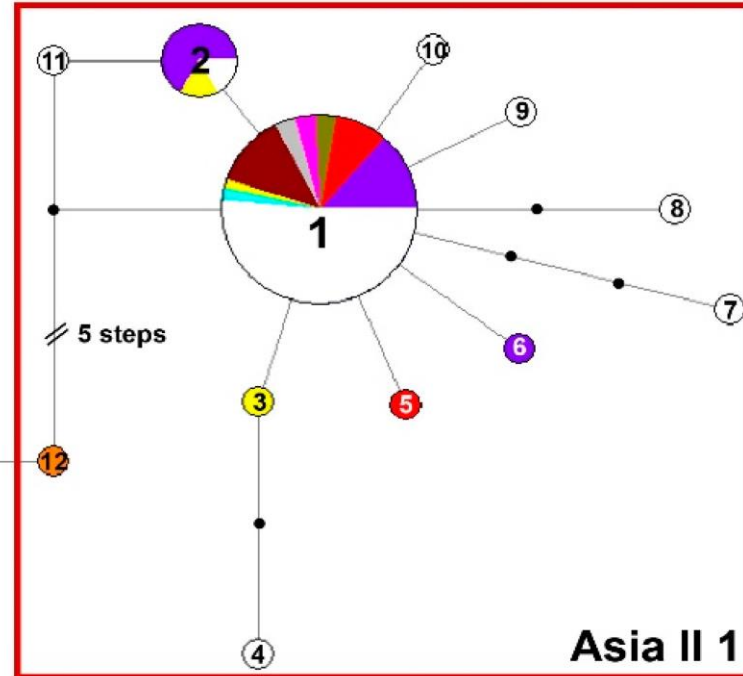
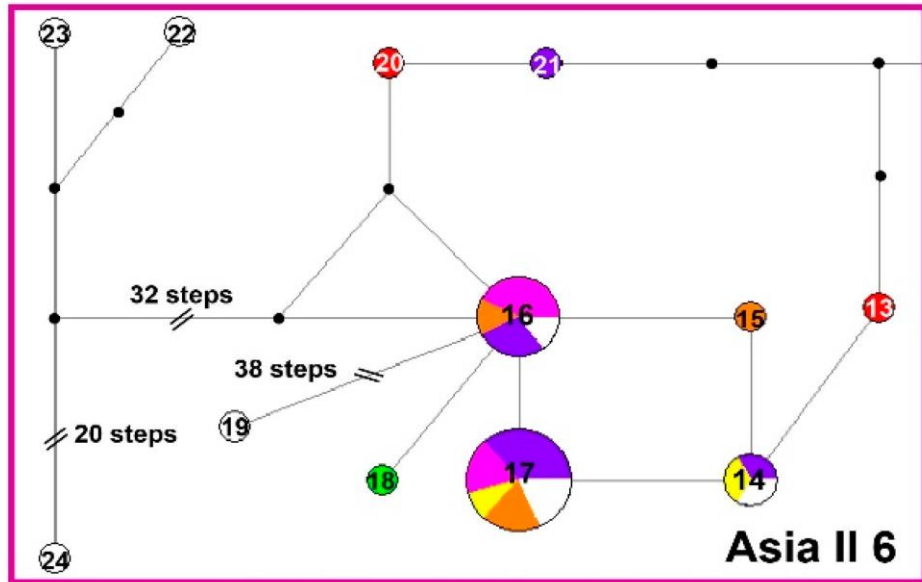


B

Cryptic Species	n	K	H
Asia II 1	37	0.727	0.417
Asia II 6	22	1.433	0.775

- A. Maximum Likelihood phylogenetic analysis of all consensus sequences from the region. mtCOI using IQTree with 1000 bootstrap replications involving 669 nt of sequences obtained in this study (in red) and reference sequences from whiteflies occurring in Asia.
- B. Distribution and frequency of cryptic species found in Lao PDR. **n** = number of sequences; **k** = average number of nucleotide differences; **H** = haplotypic diversity.

Haplotypes Network



867 bp Size of the sequence used in this study

A haplotype refers to a set of DNA variants along a single gen.

```
ACGTGATGAAATTCCTTAGGACCTATCG
ACGTGATGAAATTCGTTAGGACCTATCG
ACGTGATGAAATTCATTAGGACGTATCG
```

Haplotypes obtained from Median Joining with Network Software. Twenty-four mitochondrial cytochrome oxidase I haplotypes from 104 individuals of Asia II 1 and Asia II 6 are observed. The area of the circles is proportional to haplotype frequency in the dataset. The geographical region of each haplotype is color-coded and the small black circles indicate mutational changes.



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Mitochondrial Genetic Diversity of *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) Associated with Cassava in Lao PDR

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Thanks!