Enhanced regional diagnostic protocols, tools and information platforms fit for purpose

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Cassava Crop Protection Team Lead

Ho Chi Minh, October 2, 2023

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Overview of activities, outputs & outcomes

ACTIVITY	OUTPUTS/MILESTONES	COMMENT
Activity 3.1: Conduct training and capacity building of plant protection institutes in key diagnostic tools, sampling design, and data management platforms	A standard and **basic surveillance protocol** (BSP) in major cassava growing region in SEA Training material developed for use within the region in 2019	Regional Team: BSP validated in the field in 2019-2020.
Activity 3.2: Design, implement and communicate regional surveillance activities for CMD and CWBD in Vietnam, Cambodia, Laos, Myanmar and Thailand, with results shared in a common platform	Protocols for uploading and accessing data – Report Dec 2019 Updated **maps** with "confirmed/suspected/non-infected" data. Sampling data in Sep-Nov Yr 2,3,4	Regional team: Completed for years 2020, 2021, 2022
Activity 3.3: Understand the distribution and diversity of whitefly populations throughout the cassava production regions of Vietnam, Cambodia, Lao PDR, Myanmar and Thailand	A first regional indexed collection of cassava whiteflies Sequence **diversity of whitefly populations in SEA** identified and characterized for 2020 and 2022 Online access to SEA Whitefly Distribution maps via PestDisPlace	
Activity 3.4: Evaluate new technologies for rapid field diagnostics with particular applications in seed systems	A **LAMP** protocol for rapid detection of SLCMV evaluated in field and under minimal laboratory conditions A novel and robust **PCR** test for **CWBD** is available and validated under laboratory conditions	Jimmy Botella Ana M. Leiva, Alejandra Gil
Activity 3.5: Develop and validate protocols for screening and biological characterisation of cassava diseases, particularly CWBD	**grafting of CWB** on cassava rootstocks did not work. Vascular necrosis induced by CWBD kills the small grafts impeding transmission of the pathogen. Successful transmission of CWBD by a modified side-grafting protocol under high humidity conditions Koch's postulates	Juan Manuel Pardo Warren Arinaitwe Pinkham Vongphachanh

M
T
R

Cassava Crop Protection Team

Team Colombia: Ronald Montes, Jenyfer Jimenez**,** Alejandra Gil-Ordoñez, Viviana Dominguez, Sara Caicedo, Juan Manuel Pardo, Rafael Rodriguez, Ana Maria Leiva

Team Laos: Warren Arinaitwe**,** Khamla Xaiyavong, Chanpheng Xaymany, Latsamy Dethanonglack

Strengthening regional surveillance/dx networks

PPRI (Vietnam), NAFRI, PPC (Lao PDR), GDA (Cambodia), KU (Thailand)

COVID times

How many plants per field?

To calculate the **sample size** required to detect the set infection level for the population with the set probability

(detected means one or more in sample is infected)

required

infect

sample size

leve

30

⇑

modify

Contents lists available at ScienceDirect

Virus Research

journal homepage: www.elsevier.com/locate/virusres

Short communication

Surveillance and diagnostics of the emergent Sri Lankan cassava mosaic virus (Fam. *Geminiviridae*) in Southeast Asia

Wanwisa Siriwan[a], Jenyfer Jimenez[b], Nuannapa Hemniam[a], Kingkan Saokham[c,d], Diana Lopez-Alvarez[b], Ana M. Leiva[b], Andres Martinez[e], Leroy Mwanzia[e], Luis A. Becerra Lopez-Lavalle[f], Wilmer J. Cuellar[b,*]

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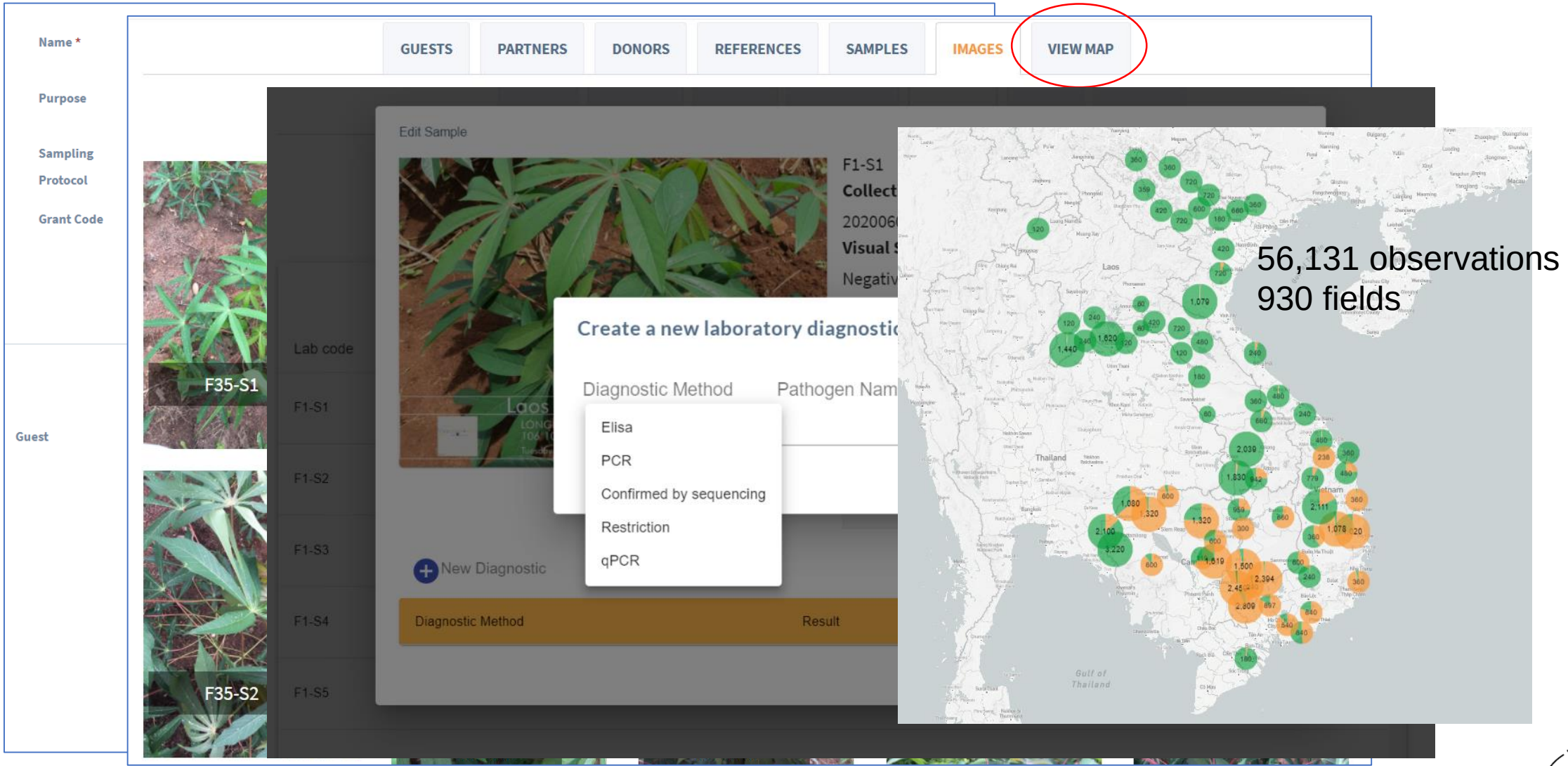
[f] *Cassava Genetics Laboratory, International Center for Tropical Agriculture (CIAT), Cali, Colombia*

PestDisPlace Projects

https://pestdisplace.org



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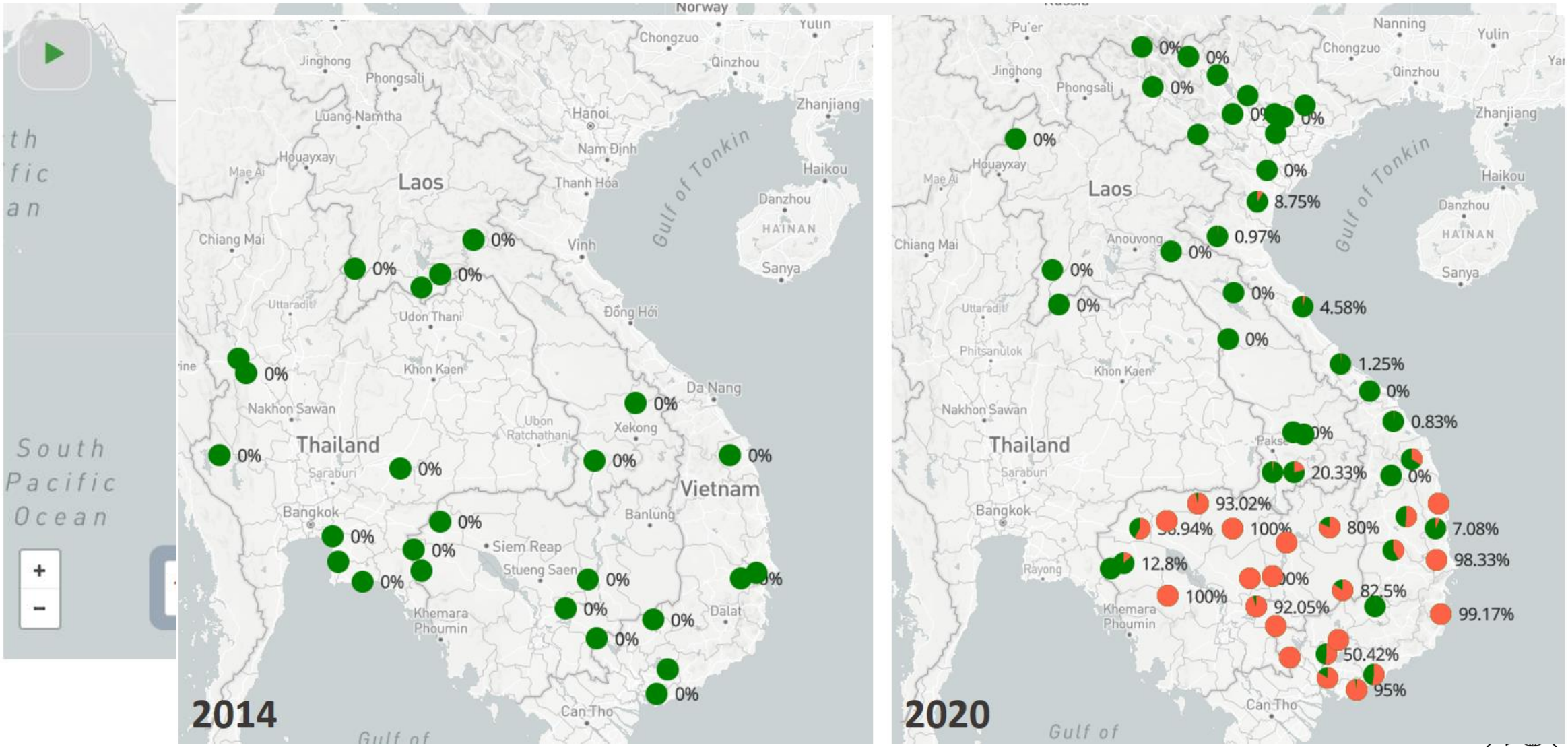
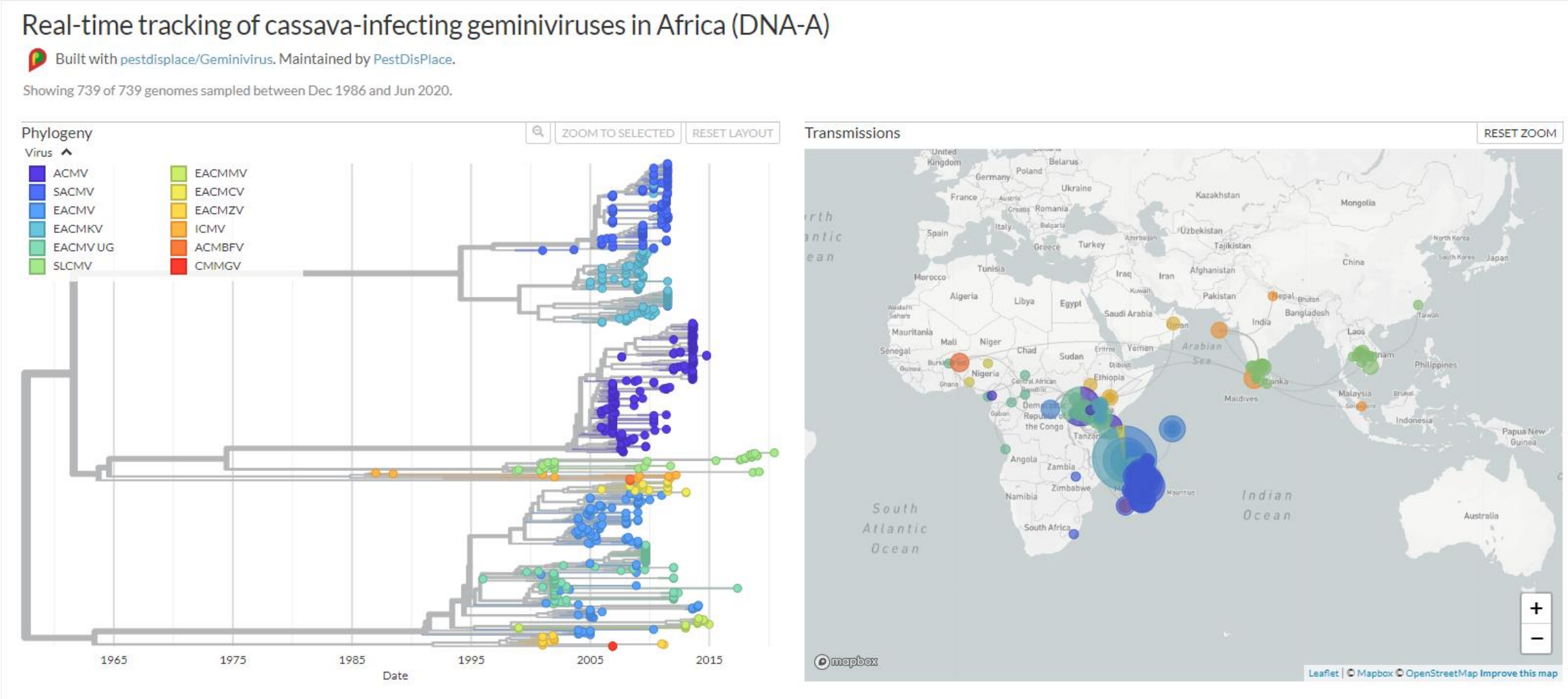
Baseline comparative incidences



12 species of viruses causing CMD



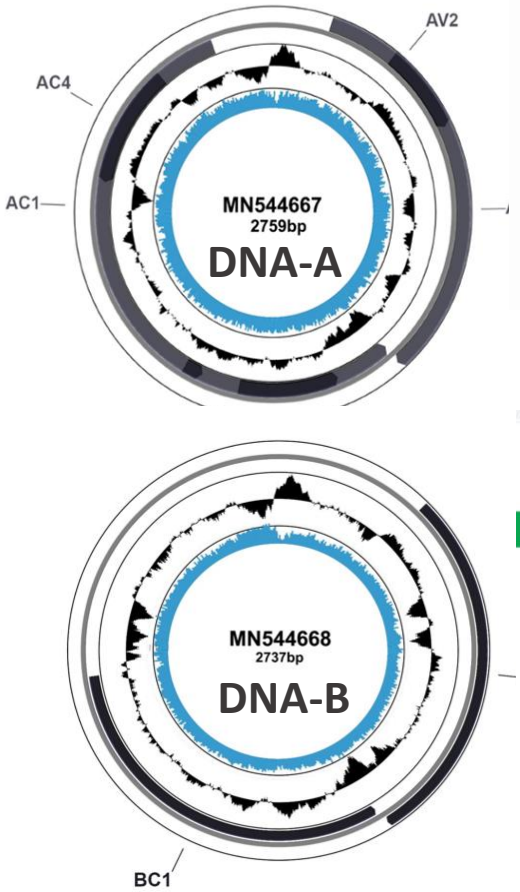
How can we detect all related begomoviruses in SEA at once

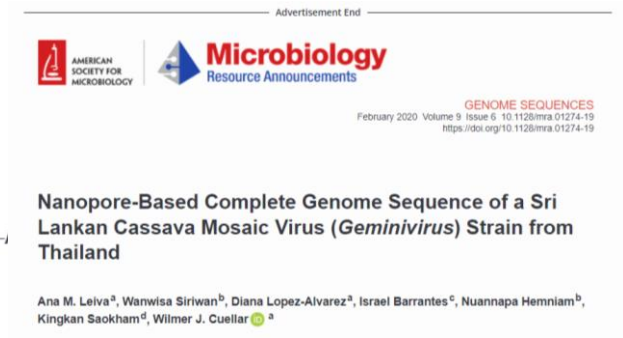
A generic method targeting all circular viral DNA

Rolling Circle Amplification using Phi29 using Random hexamer primers

Nanopore

Genome analysis





Advertisement End

American Society for Microbiology | Microbiology Resource Announcements

GENOME SEQUENCES

February 2020 Volume 9 Issue 6 10.1128/mra.01274-19 https://doi.org/10.1128/mra.01274-19

Nanopore-Based Complete Genome Sequence of a Sri Lankan Cassava Mosaic Virus (*Geminivirus*) Strain from Thailand

Ana M. Leiva[a], Wanwisa Siriwan[b], Diana Lopez-Alvarez[a], Israel Barrantes[c], Nuannapa Hemniam[b], Kingkan Saokham[d], Wilmer J. Cuellar[a]

APS Publications

Plant Disease Home About Submit Journals Books Publisher's Home

DISEASE NOTES OPEN

First Report of Cassava Mosaic Disease and Sri Lankan Cassava Mosaic Virus in Laos

K. Chittarath, J. Jimenez, P. Vongphachanh, A. M. Leiva, S. Sengsay, D. Lopez-Alvarez, T. Bounvilayvong, D. Lourido, V. Vorlachith, and W. J. Cuellar

Affiliations

Published Online: 23 Apr 2021 | https://doi.org/10.1094/PDIS-09-20-1868-PDN

Only one begomovirus species found in SEA so far

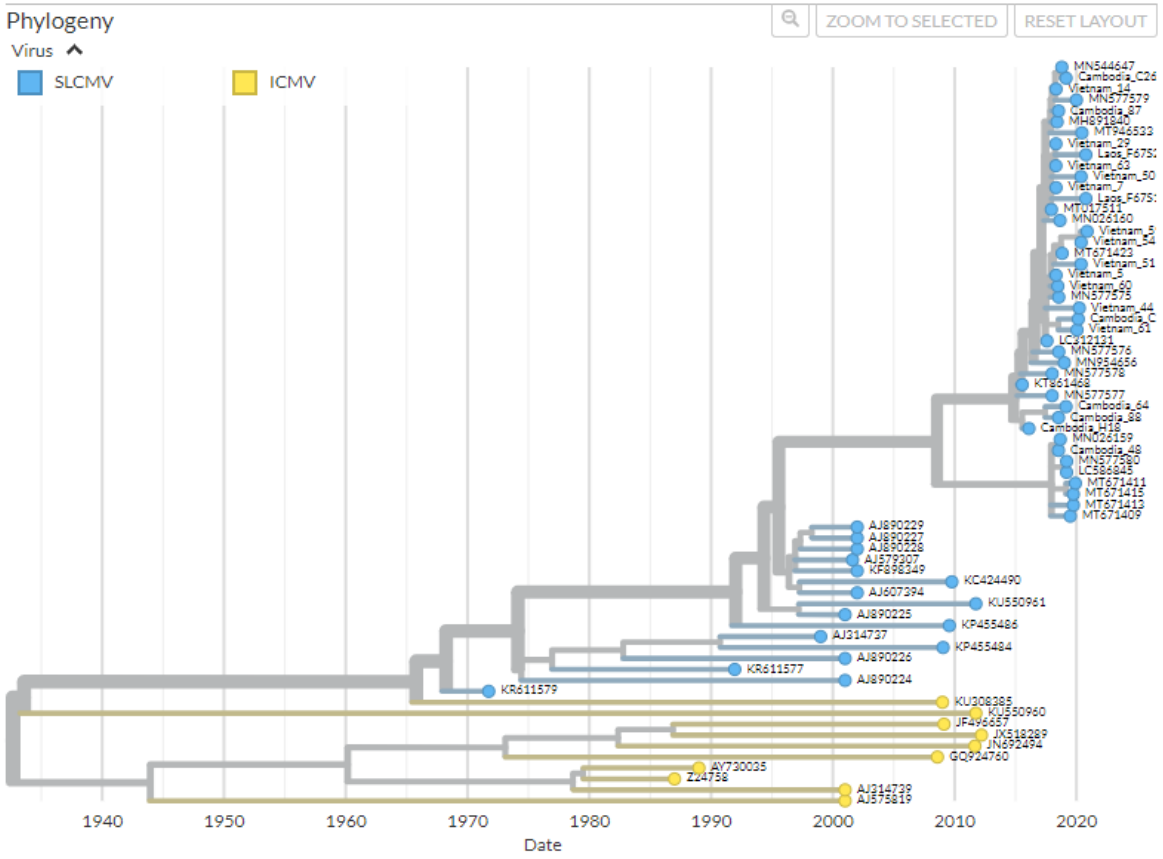
Alianza Bioversity & CIAT CGIAR

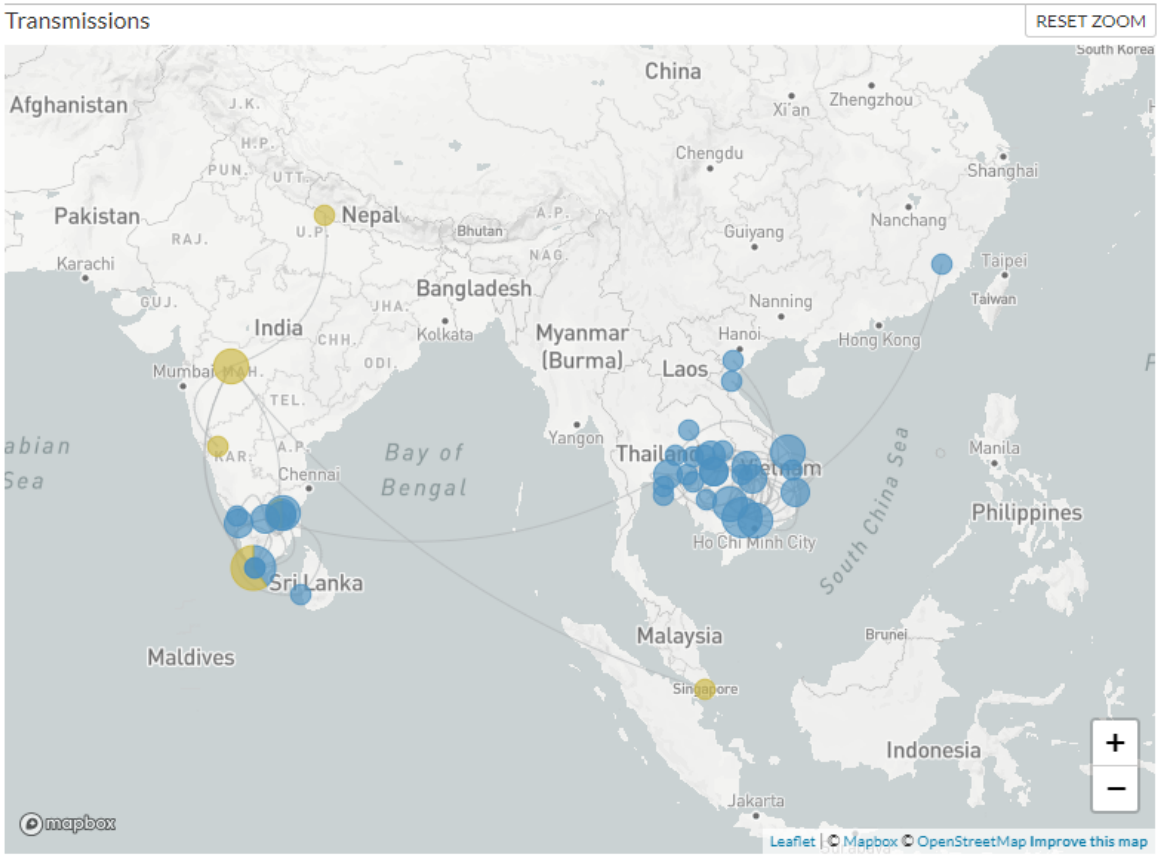
Genomic surveillance of SLCMV in SEA

Real-time tracking of cassava-infecting geminiviruses in Asia (DNA-A)

Built with pestdisplace/CMDASIA1. Maintained by PestDisPlace.

Showing 68 of 68 genomes sampled between Sep 1971 and Nov 2020.

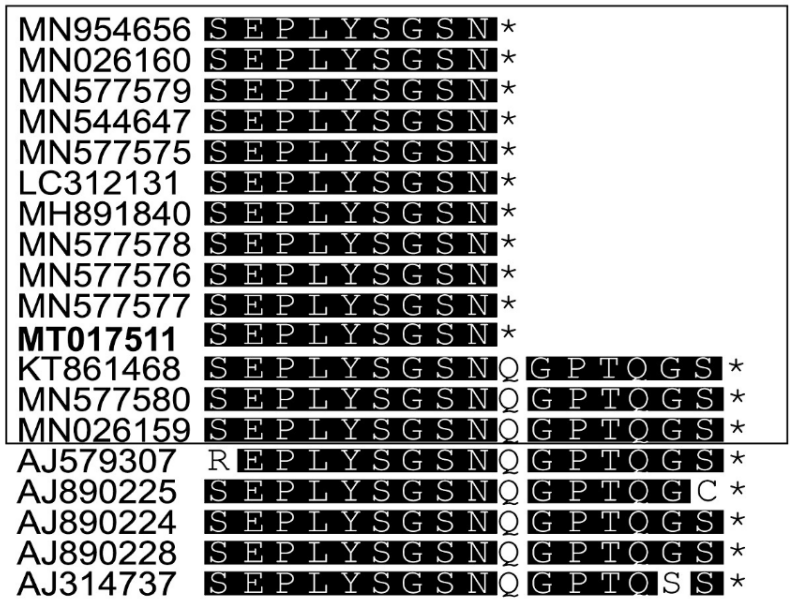




Nucleotide diversity of genome

RESET LAYOUT | ENTROPY EVENTS

Sri Lankan cassava mosaic virus

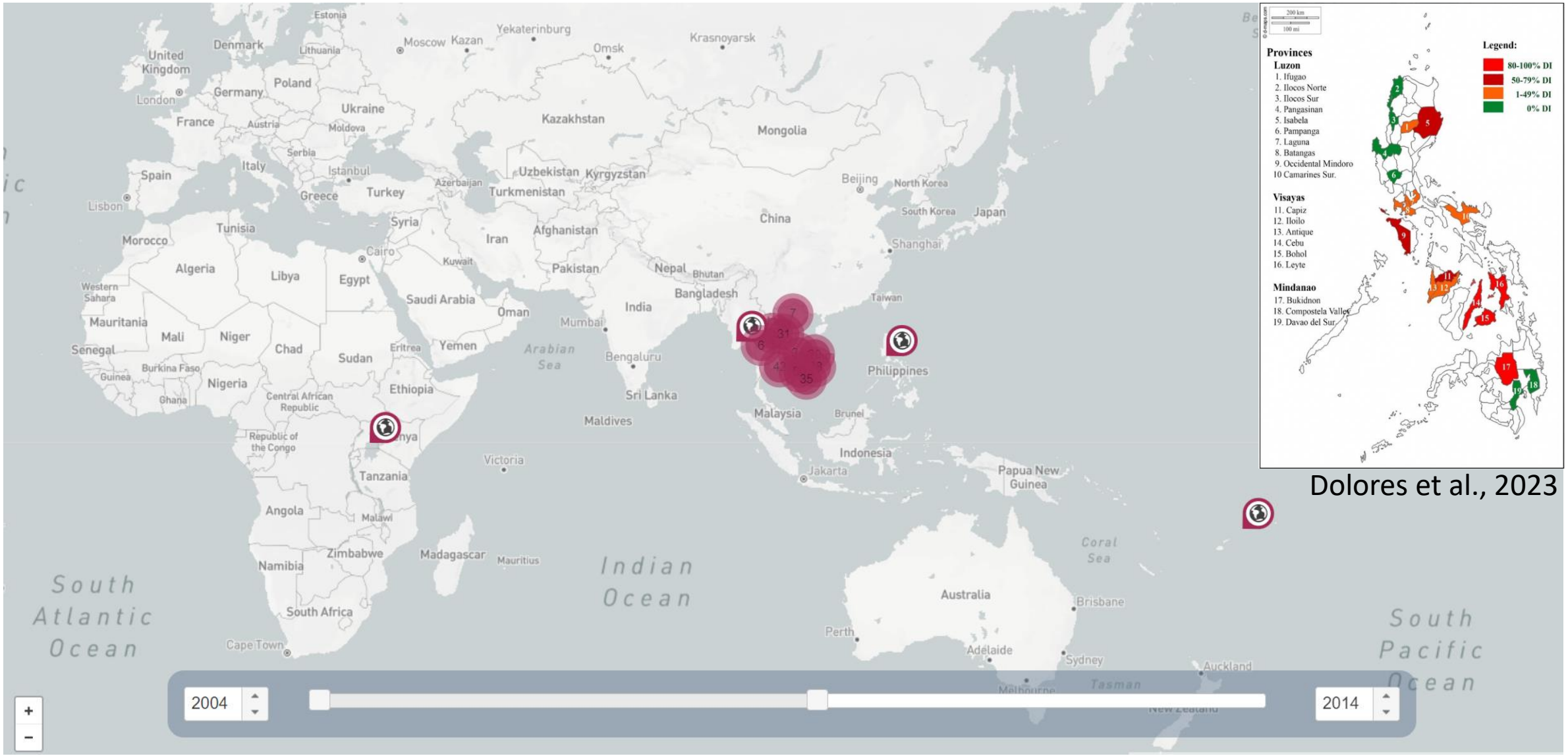


Two versions of the *rep* gene

CMD in SEA emerged after a ‘first wave’ of CWBD

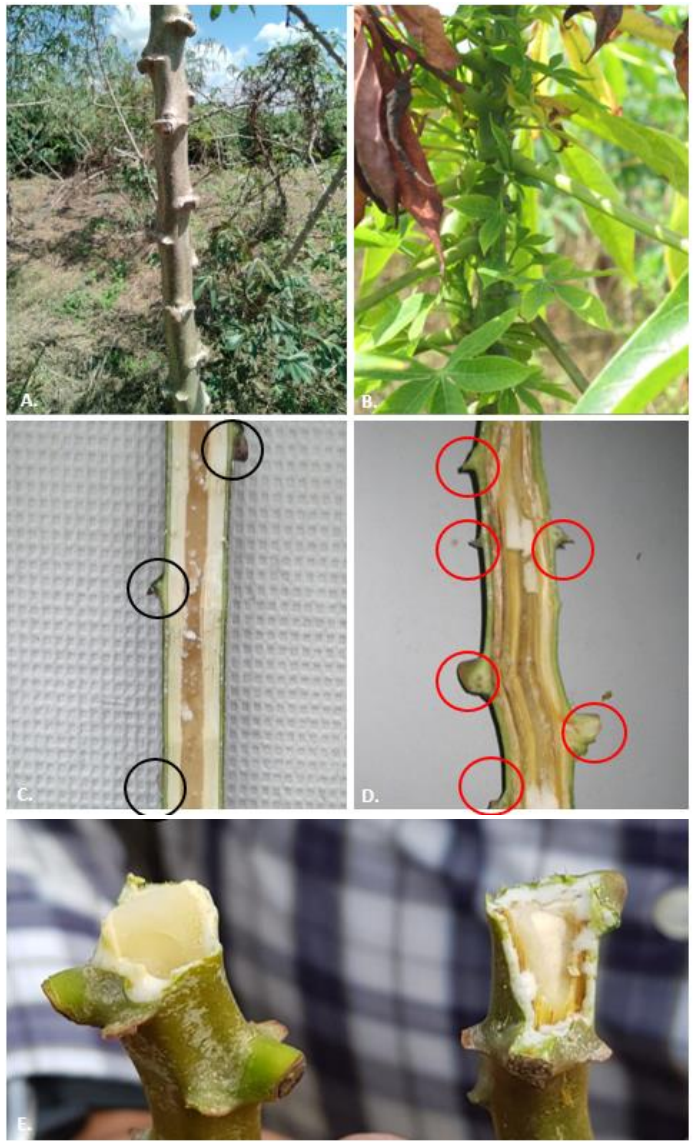
- 2010: CWBD incidence ~80% in Yen Bai, Quang Ngai and Dong Nai (north, central and south Vietnam)
- 2012: CWBD incidences of 30-40% in Cambodia in the provinces of Kampong Cham, Kratie and Prey Veng.
- 2012: field surveys in Chachoengsao and Rayong in southern Thailand, report similar “high incidences” of CWBD.
- 2014: average incidence of CWBD in SEA 32% (highest in Cambodia, 46%)

Global distribution of CWBD (Symptoms) reports



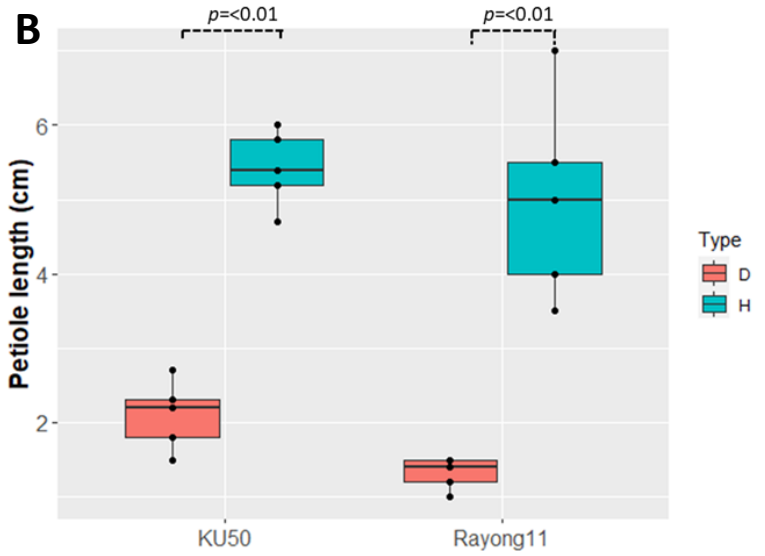
Dolores et al., 2023

Symptoms can be transmitted by grafting

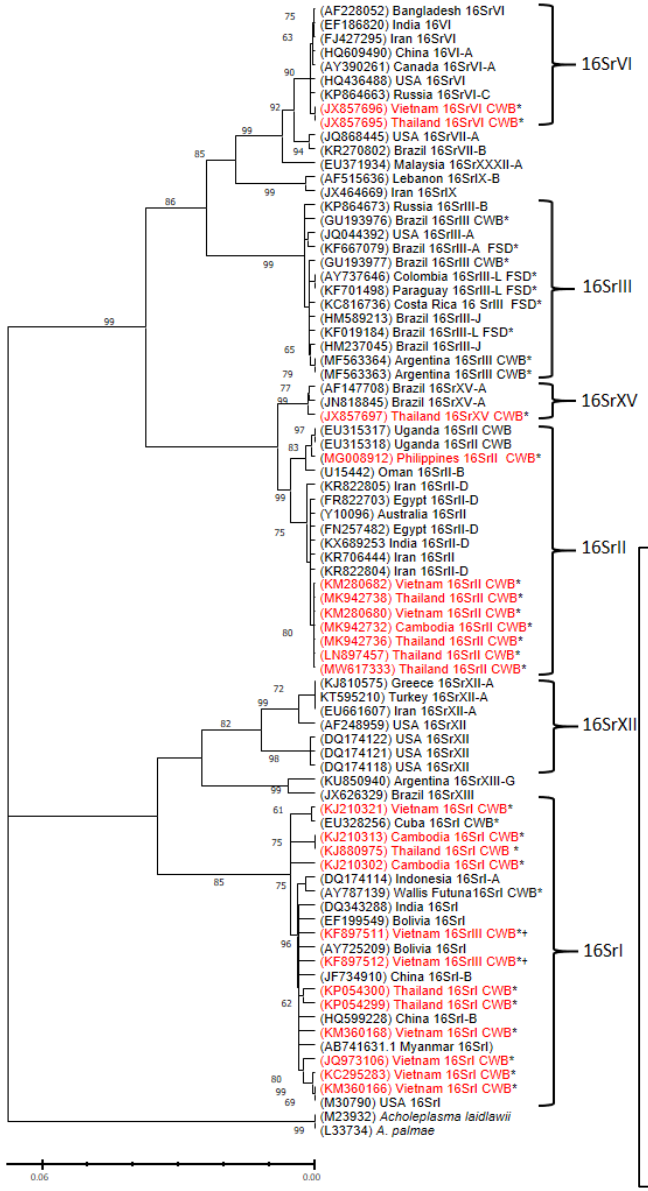


A

B



Phytoplasma is not associated with CWBD



Phenotype	PCR (-)	PCR (+)	Total
Healthy	427	17	444
Classic witches' broom symptoms	12	22	34

plants

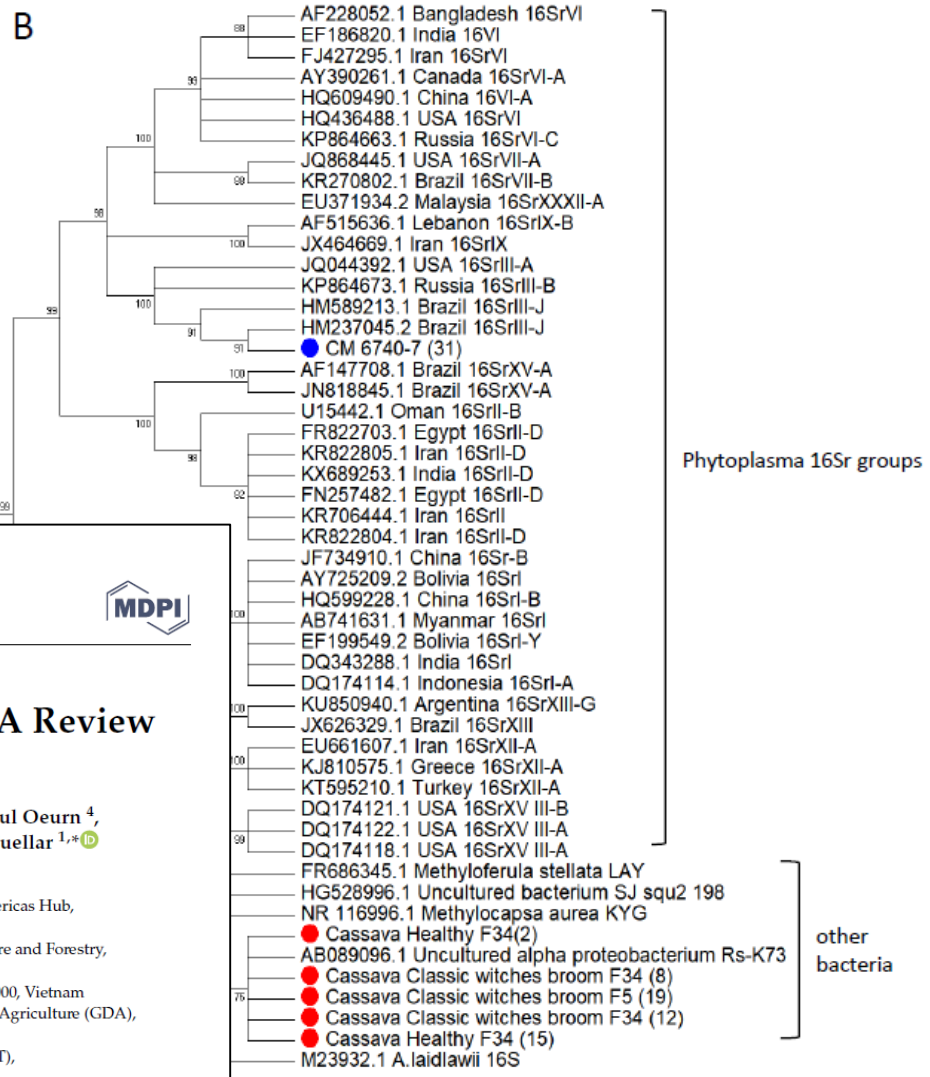
MDPI

Review

Cassava Witches' Broom Disease in Southeast Asia: A Review of Its Distribution and Associated Symptoms

Juan M. Pardo [1], Khonesavanh Chittarath [2], Pinkham Vongphachanh [2], Le Thi Hang [3], Samoul Oeurn [4], Warren Arinaitwe [5], Rafael Rodriguez [1], Sok Sophearith [6], Al Imran Malik [5] and Wilmer J. Cuellar [1,*]

1 Cassava Program, International Center for Tropical Agriculture (CIAT), The Americas Hub, Km 17 Recta Cali-Palmira, Cali 763537, Colombia
2 Plant Protection Center (PPC), Department of Agriculture, Ministry of Agriculture and Forestry, Vientiane P.O. Box 811, Laos
3 Plant Protection Research Institute (PPRI), Duc Thang, Bac Tu Liem, Ha Noi 100000, Vietnam
4 Plant Protection Sanitary and Phytosanitary Department, General Directorate of Agriculture (GDA), Phnom Penh 120406, Cambodia
5 Cassava Program Asia Office, International Center for Tropical Agriculture (CIAT), Vientiane P.O. Box 783, Laos
6 Cassava Program Cambodia Office, International Center for Tropical Agriculture (CIAT), Phnom Penh 120904, Cambodia
* Correspondence: w.cuellar@cgiar.org



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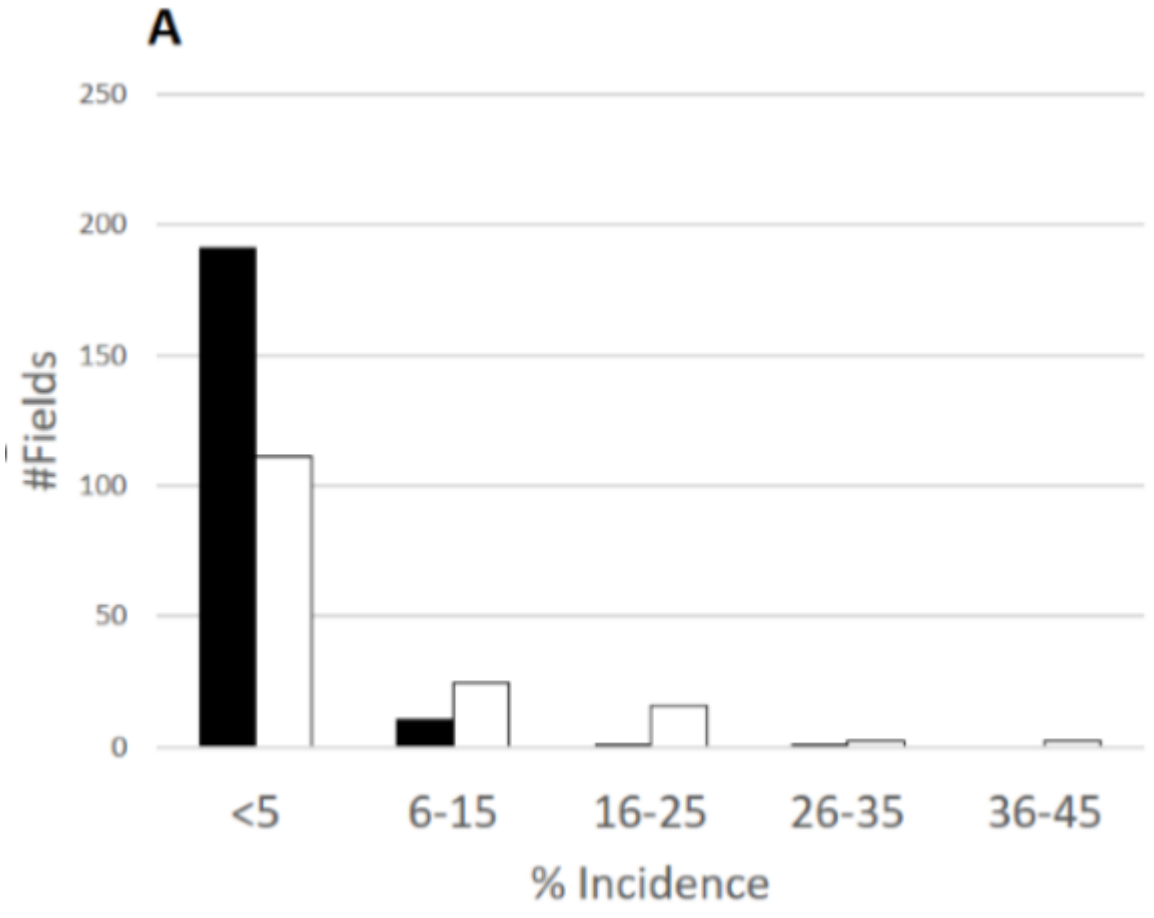
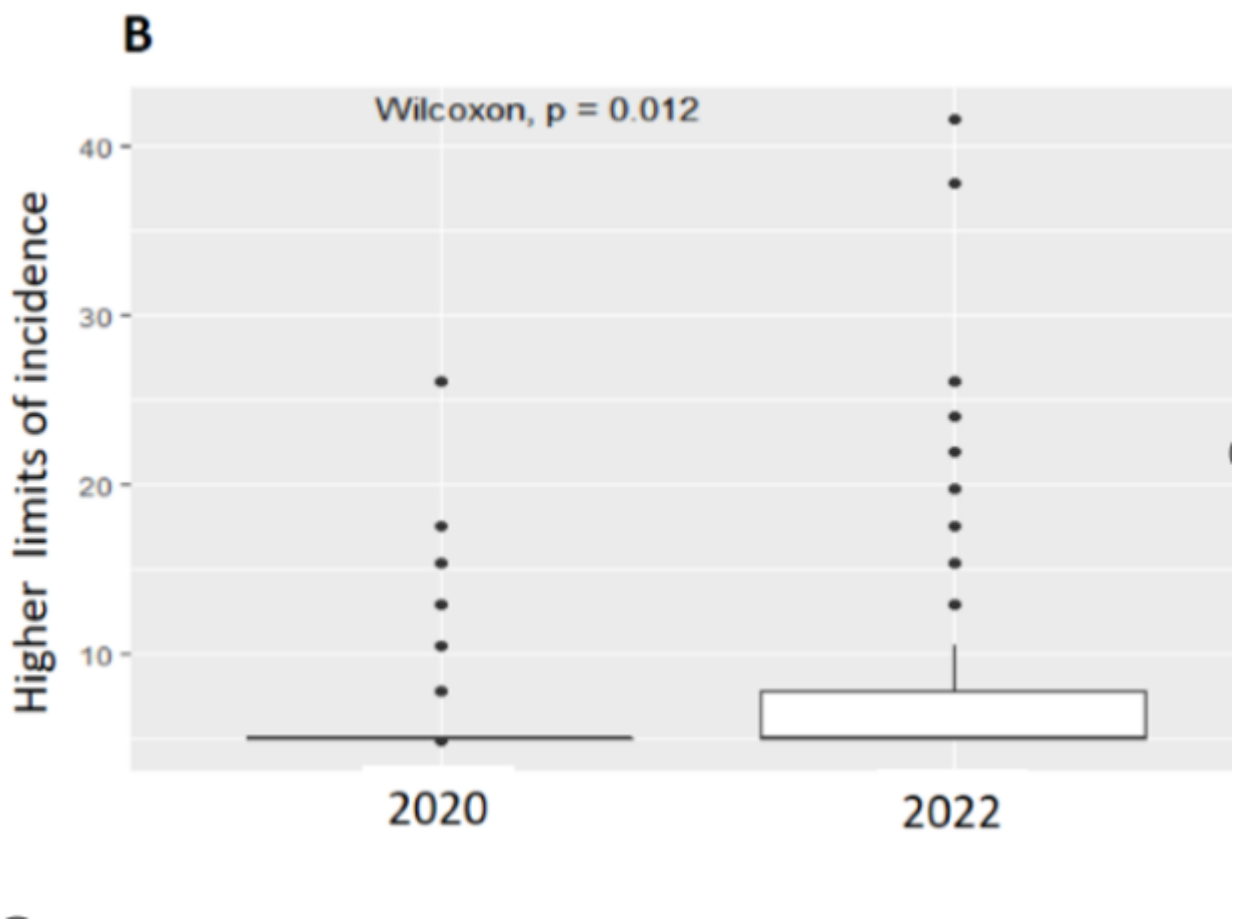
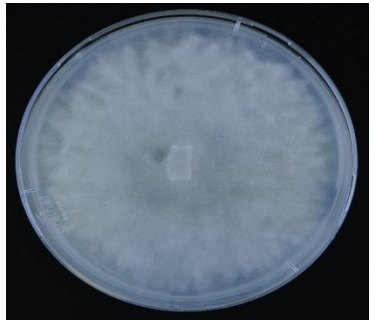
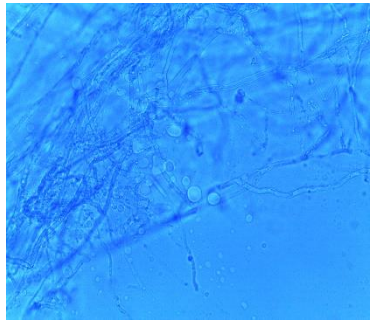
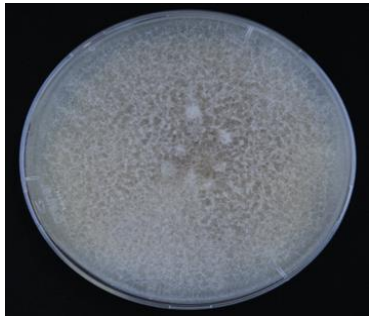
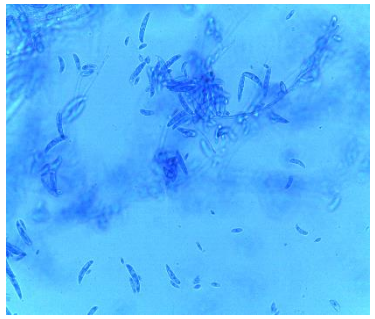
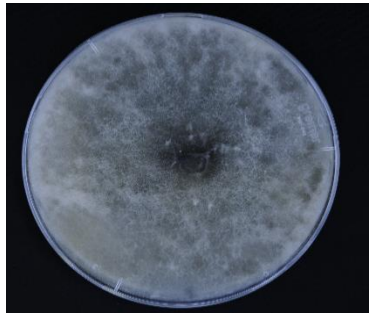
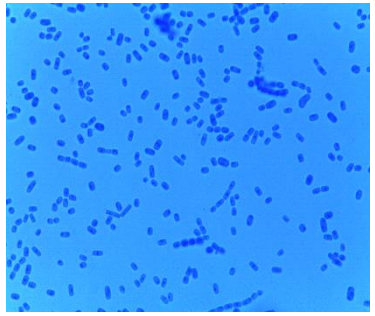
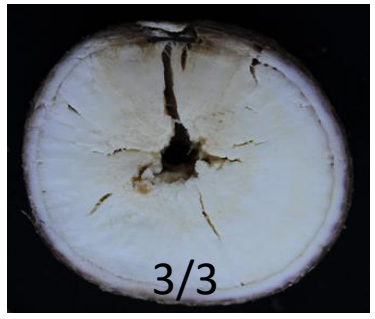
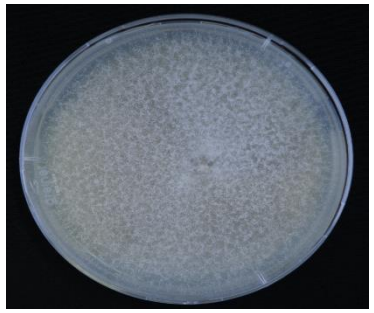
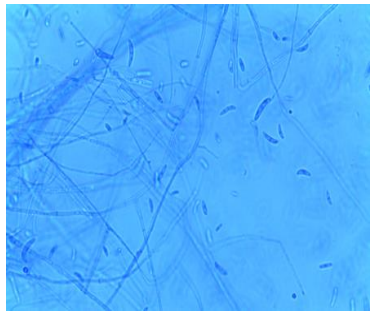
CWBD incidence is increasing in LAO, VTN and KHM





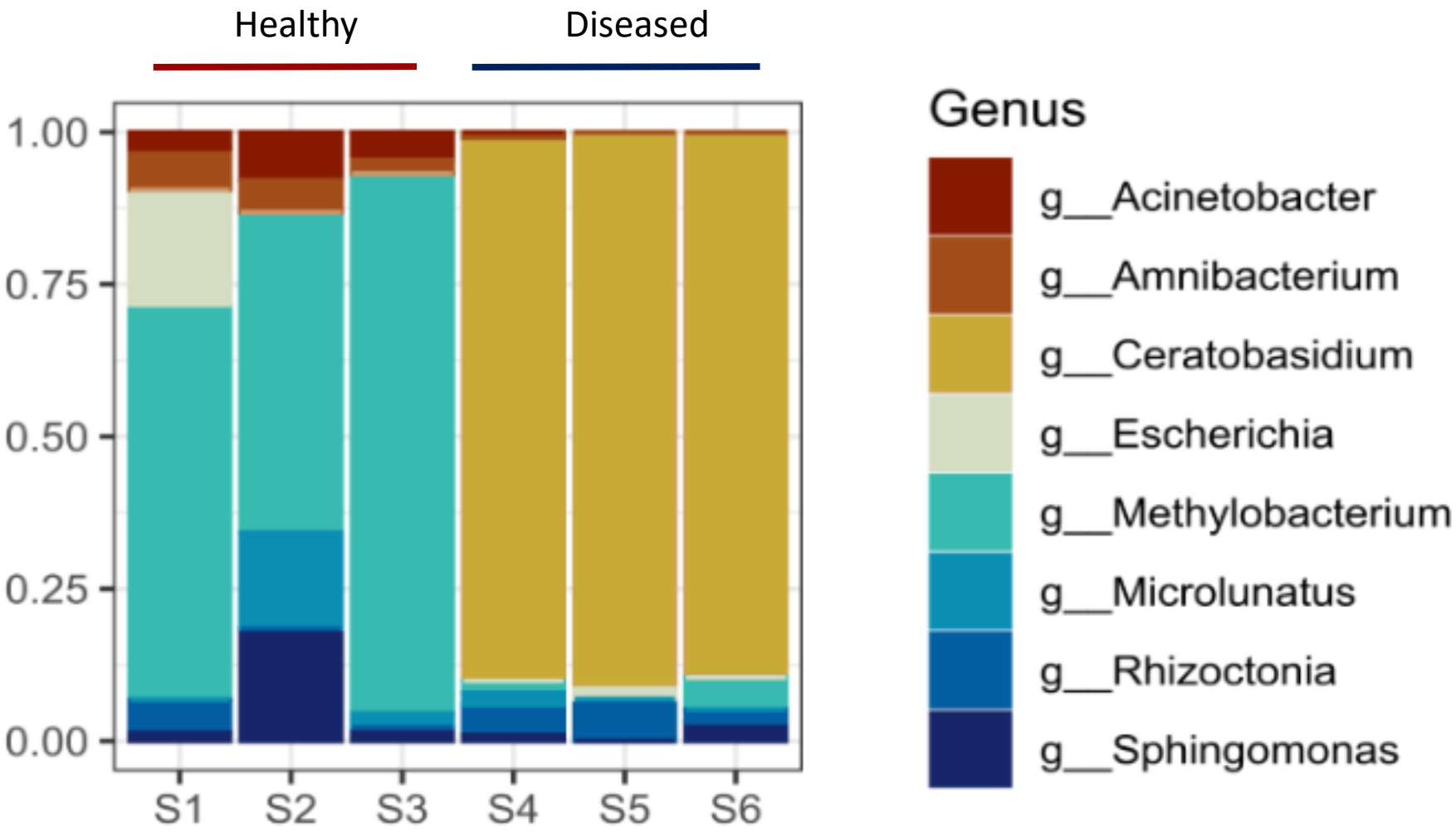
Root rot

MCOL1505

	Symptoms	*in vitro*	Microscopy	Indicator	Control
Phytophthora spp.					
Fusarium spp.					
Neoscytalidium spp.					
Fungi isolated from symptomatic roots producing no symptoms in the indicator plant					

Can we have a generic look at the microbial content of CWBD?

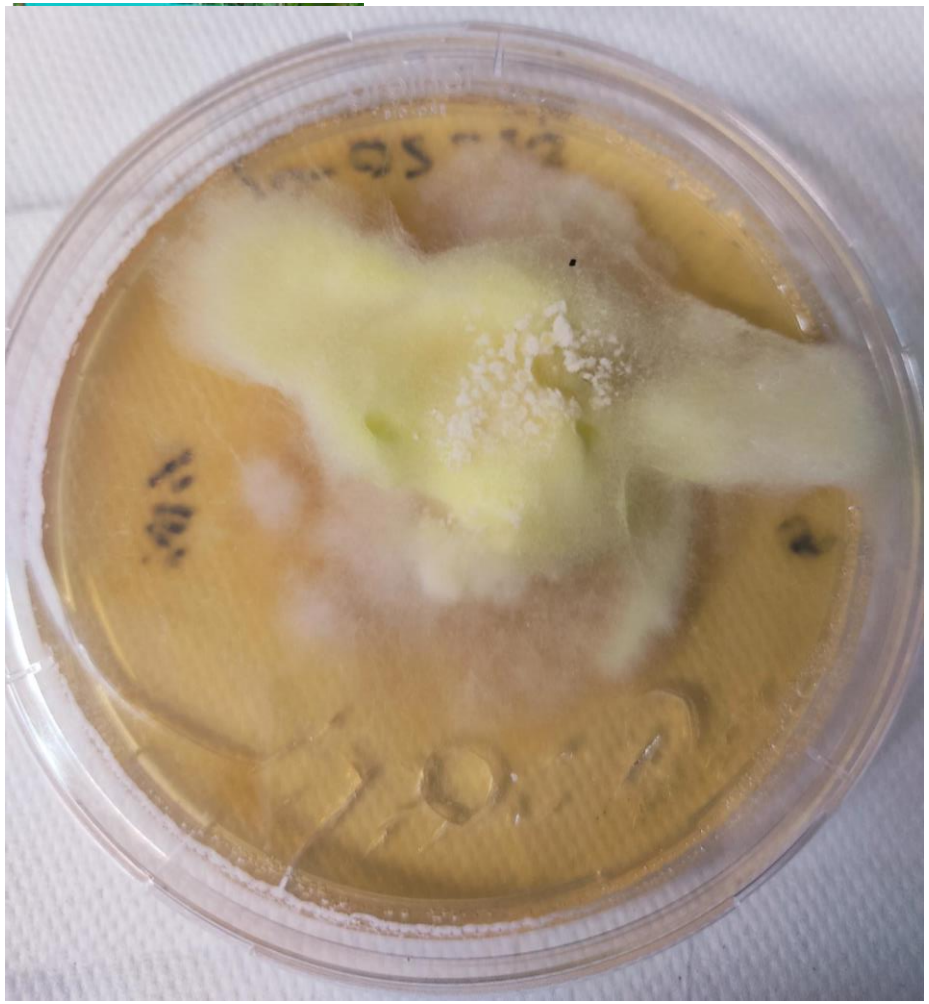
First metagenomic analysis of CWBD

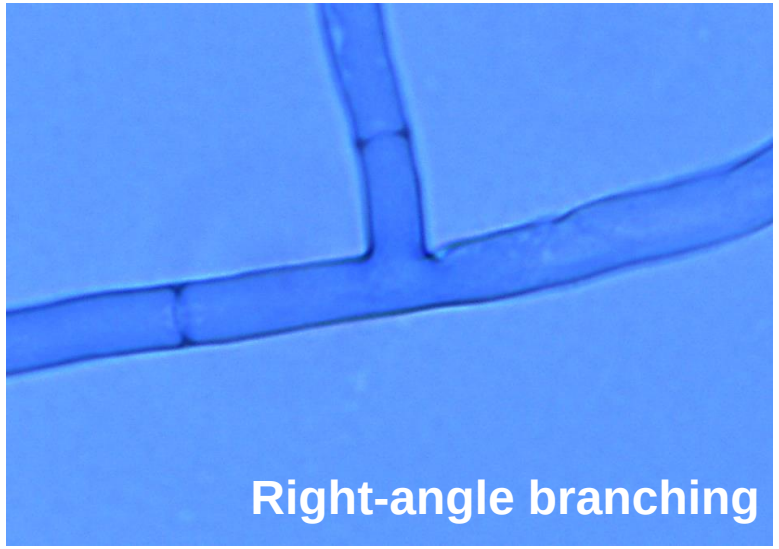


Summary of sequencing results

Sample code*	Total Reads	% Reads mapped to cassava	# Reads unmapped	No. of assigned contigs	Avg size (bp)	N50 (bp)	Ceratobasidium contigs
Healthy 1	196,515,051	97.95%	4,514,055	1,401	679	726	0
Healthy 2	250,533,683	98.48%	4,184,629	1,839	650	689	0
Healthy 3	243,291,842	97.93%	5,506,137	3,596	613	635	0
CWBD 4	214,214,660	98.20%	4,232,419	26,668	674	761	16712
CWBD 5	218,249,943	98.51%	3,581,978	9,427	567	587	5528
CWBD 6	211,118,163	93.25%	15,730,655	35,765	992	3,754	19734

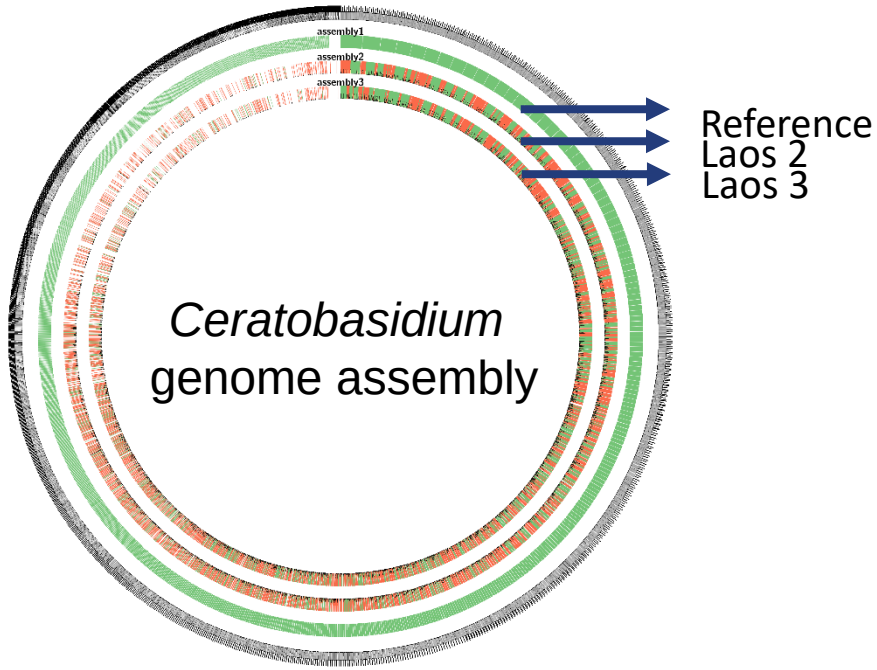
Ceratobasidium sp. in plants with CWBD



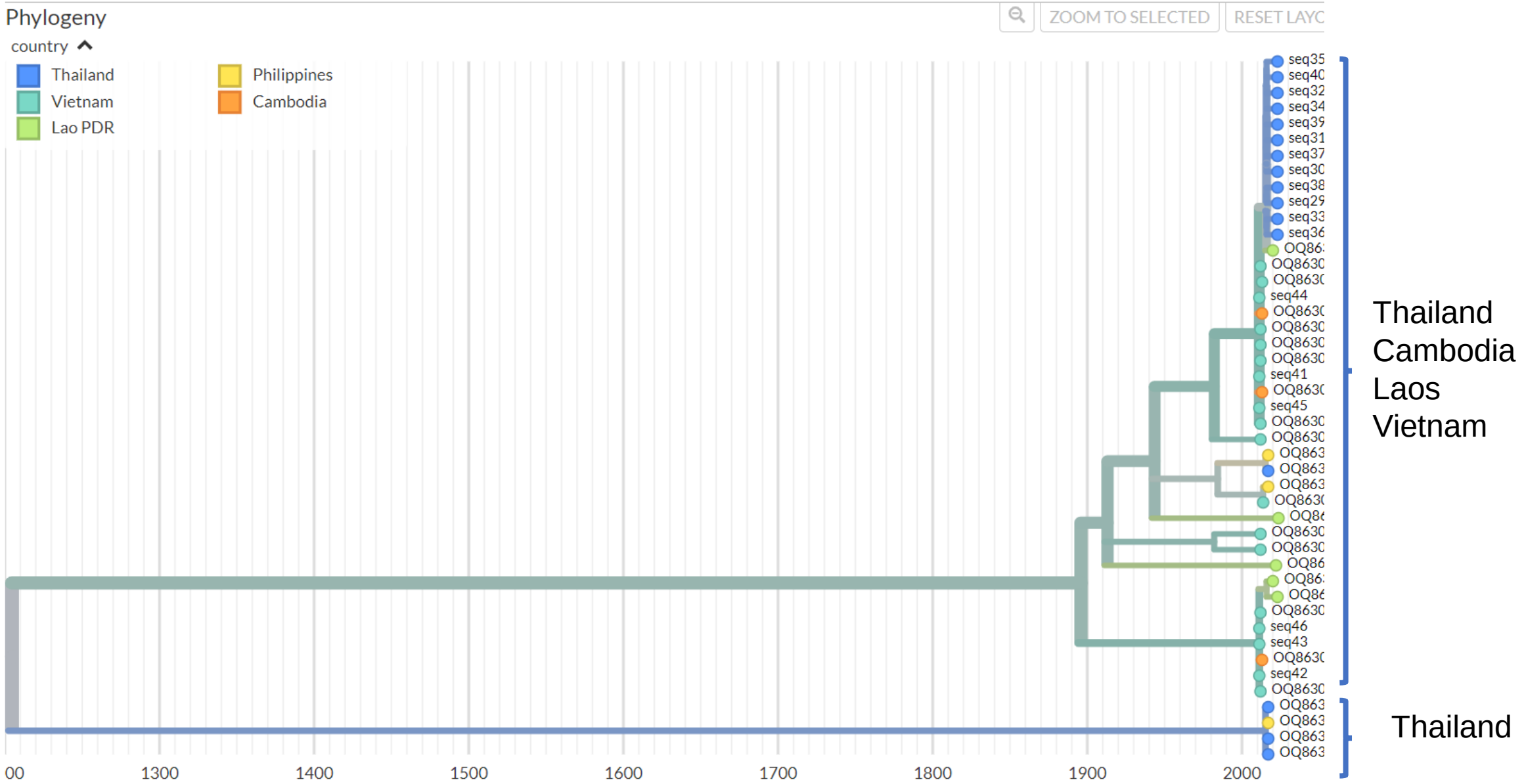


Developing a reliable molecular diagnostic test for CWBD

	CWBD		
Phenotype	**No of samples**	**PCR (-)**	**PCR (+)**
Healthy	123	122	**1**
Diseased	41	1	**40**
Other symptoms	3	0	0
Unclear symptoms	4	0	0
Total		**171**	
Sensitivity		97.6%	
Specificity		99.2%	



Ceratobasidium sp in CWBD plants (preliminary)



Outcomes

The research outcomes continue during the reporting period with several activities reaching publication and new knowledge shared with stakeholders. These include:

- Multiyear regional surveillance maps (CMD, CWBD, WF)
- Diagnostics tools: generic, specific, low-cost
- Identification of only SLCMV and low abundance of *B. tabaci*
- Identification and isolation of the causal agent of CWBD for screening studies
- 5 scientific publications (open access) reporting these findings

Thanks!