

Molecular identification of mealybug species (Hemiptera: Pseudococcidae) affecting *Theobroma cacao* for improved pest management

Alina Puig ^{1,*}



Sarah Wurzel ¹



Stephanie Suarez ²



Jerome Niogret ³



Jean-Phillipe Marelli ⁴



¹ Subtropical Horticultural Research Station, USDA-ARS, Miami FL 33158 USA

² Mars Inc., 13601 Old Cutler Road, Miami FL 33158 USA

³ Mars Inc., James Cook University, Smithfield QLD 4878 Australia

⁴ Mars Plant Sciences Laboratory, Davis CA 95616 USA

* Correspondence: alina.puig@usda.gov

IECE
2021

The 1st International Electronic
Conference on Entomology

01-15 JULY 2021 | ONLINE

Economic impact of mealybugs on crops

- Mealybugs (Hemiptera: Pseudococcidae) feed on sap and reduce plant vigor
- On cacao, their primary economic significance of is the ability to transmit viruses
- Most virus affecting cacao belong to the *Badnavirus* genus and are transmitted by mealybugs

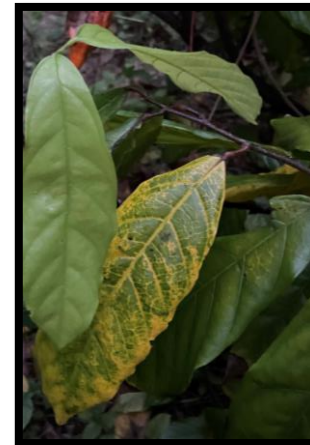
Cacao swollen shoot virus (CSSV)

Location: West Africa



Cacao mild mosaic virus (CaMMV)

Location: The Americas



Species identification for effective control

- Effective management depends on accurate identification of species present
- Even closely related species have distinct life cycles and are vulnerable to different biological control organisms
- For example: a study on the parasitoid *Anagyrus sinope* found it could only parasitize one of the five *Pseudococcus* species tested (Chong & Oetting 2007)



(a) Mealybugs and eggs on pods

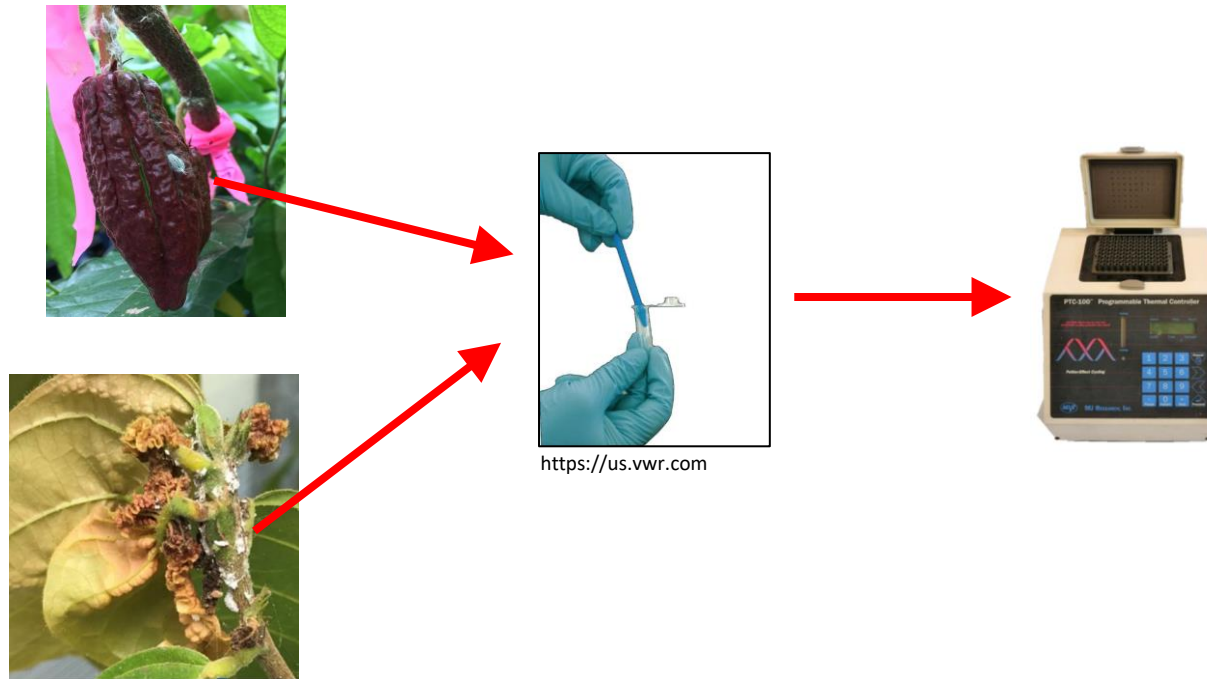
(b) leaf distortion characteristic of feeding by *Maconellicoccus hirsutus*.

Rationale

- Due to the high diversity of Pseudococcidae, each region has different species composition
- Morphological differentiation is challenging for non-specialists
- Sequence-based identification reduces the reliance on delicate morphological features



Methods: DNA extraction



- Mealybugs collected from cacao trees in Miami, FL
- DNA extracted from individual specimens



Methods: Markers and primers

Gene	Primers	Sequence (5'-3')	Amplicon Size	Reference
COI	MFCOI MRCOI	ATATCTCAAATTATAAATCAAGAA ATTACACCTATAGATAAAACATAATG	379 bp	Wetten et al. 2016
ITS2	ITS2-M-F ITS2-M-R	CTCGTGACCAAAGAGTCCTG TGCTTAAGTTCAGCGGGTAG	~800 bp	Malausa et al. 2011
28S	D10F D10R	GTAGCCAAATGCCTCGTCA CACAATGATAGGAAGAGCC	738-767 bp	Dietrich et al. 2001
28S	D2F D2R	AGAGAGAGTTCAAGAGTACGTG TTGGTCCGTGTTTCAAGACGGG	310-356 bp	Belshaw & Quicke 1997; Malausa et al. 2011

Results

- BLASTn results for COI, ITS2, 28S (D10F/D10R) sequences
- Species were determined based on matches obtained with COI sequences
- **Red font** denotes incorrect organisms among top matches

	Marker	Seq. Length	Genbank match	Accession No.	% Ident.	% Coverage
<i>Pseudococcus comstocki</i>	COI	371 bp	<i>P. comstocki</i>	LC121496.1	98.9	100
	ITS2	643 bp	<i>P. comstocki</i>	KU499509.1	96.3	100
	28S	840 bp	<i>P. comstocki</i>	JF965413.1	99.8	98
<i>Pseudococcus jackbeardsleyi</i>	COI	370 bp	<i>P. jackbeardsleyi</i>	KJ187489.1	99.5	100
	ITS2	679 bp	<i>Pseudococcus viburni</i>	KF819654.1	79.2	90
	28S	801 bp	<i>Pseudococcus viburni</i>	AY427376.1	99.1	99
			<i>Oracella acuta</i>	JF965418.1	98.9	99
		<i>P. jackbeardsleyi</i>	EU188510.1	99.9	95	
<i>Maconellicoccus hirsutus</i>	COI	374 bp	<i>M. hirsutus</i>	MK090645.1	100	100
	ITS2	755 bp	<i>M. hirsutus</i>	KU883603.1	99.5	98
	28S	808 bp	<i>M. hirsutus</i>	AY427403.1	99.5	96

Parasitoid sequences obtained with primers D2F/D2R

- Encyrtid parasitoid sequences were obtained from host DNA using D2F/D2R primers
- These primers are not recommended for mealybug identification

ID	Species	Host	Origin	Collected	GenBank #
MB5	<i>Anagyrus sp.</i>	<i>Pseudococcus jackbeardsleyi</i>	USA	Jan-2021	MZ265304
MB7	<i>Anagyrus sp.</i>	<i>P. jackbeardsleyi</i>	USA	Jan-2021	MZ265305
MB8	<i>Anagyrus sp.</i>	<i>P. comstocki</i>	USA	Jan-2021	MZ265306
MB16	<i>Anagyrus kamali</i>	<i>Maconellicoccus hirsutus</i>	USA	Jan-2021	MZ265307

Conclusion

- Accurate identification of species present is essential for the selection of biological control organisms
- Routine use requires high-quality reference libraries against which sequences can be compared
- Markers **COI and ITS2** are recommended for mealybug identification
- The species detected here are widespread and polyphagous, thus the tools are useful for multiple crops and locations
- These protocols can be used by agricultural inspectors and scientists to identify mealybug specimens and study pest populations

*In this study, ITS2 was not informative for *P. jackbeardsleyi*, due to the absence of reference sequences in GenBank. However, the sequences from this study were deposited in GenBank, making this marker valuable for future research.*



Thank you

- Belshaw, R.; Quicke, D.L. A molecular phylogeny of the Aphidiinae (Hymenoptera: Braconidae). *Molecular phylogenetics and evolution* 1997, 7, 281-293.
- Chong, J.-H.; Oetting, R.D. Specificity of *Anagyrus* sp. nov. nr. *sinope* and *Leptomastix dactylopii* for six mealybug species. *BioControl* 2007, 52, 289-308.
- Dietrich, C.; Rakitov, R.; Holmes, J.; Black IV, W. Phylogeny of the major lineages of Membracoidea (Insecta: Hemiptera: Cicadomorpha) based on 28S rDNA sequences. *Molecular phylogenetics and evolution* 2001, 18, 293-305.
- Malausa, T.; Fenis, A.; Warot, S.; Germain, J.F.; Ris, N.; Prado, E.; Botton, M.; Vanlerberghe-Masutti, F.; Sforza, R.; Cruaud, C. DNA markers to disentangle complexes of cryptic taxa in mealybugs (Hemiptera: Pseudococcidae). *Journal of applied Entomology* 2011, 135, 142-155.
- Wetten, A.; Campbell, C.; Allainguillaume, J. High-resolution melt and morphological analyses of mealybugs (Hemiptera: Pseudococcidae) from cacao: tools for the control of Cacao swollen shoot virus spread. *Pest management science* 2016, 72, 527-533.