

# Genomics for the Pacific: Coconut Rhinoceros Beetle and its biocontrol agent *Oryctes nuditarsis*



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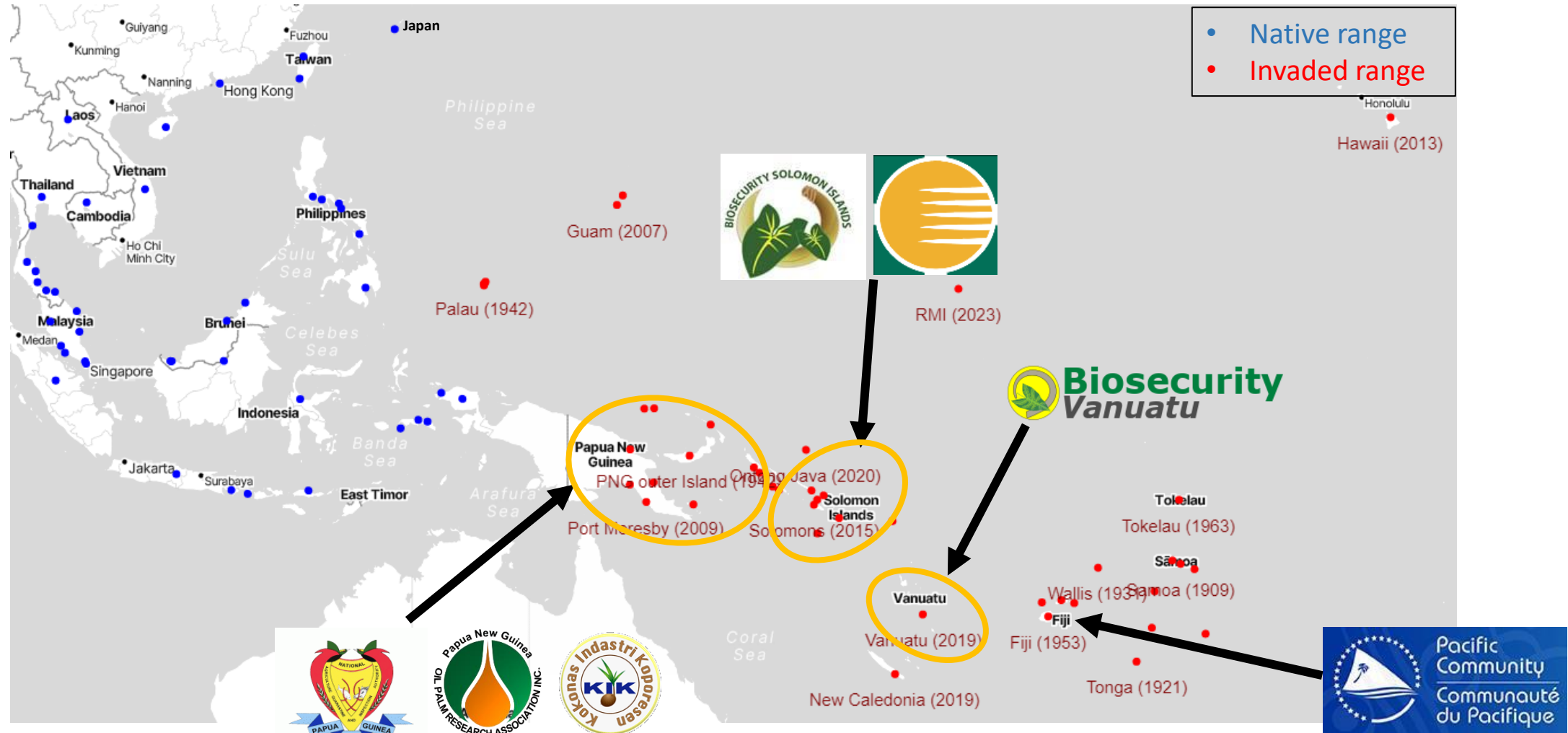
**Programme partners:**



# CRB damage



# The bigger picture

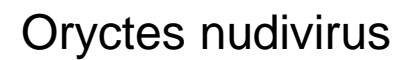




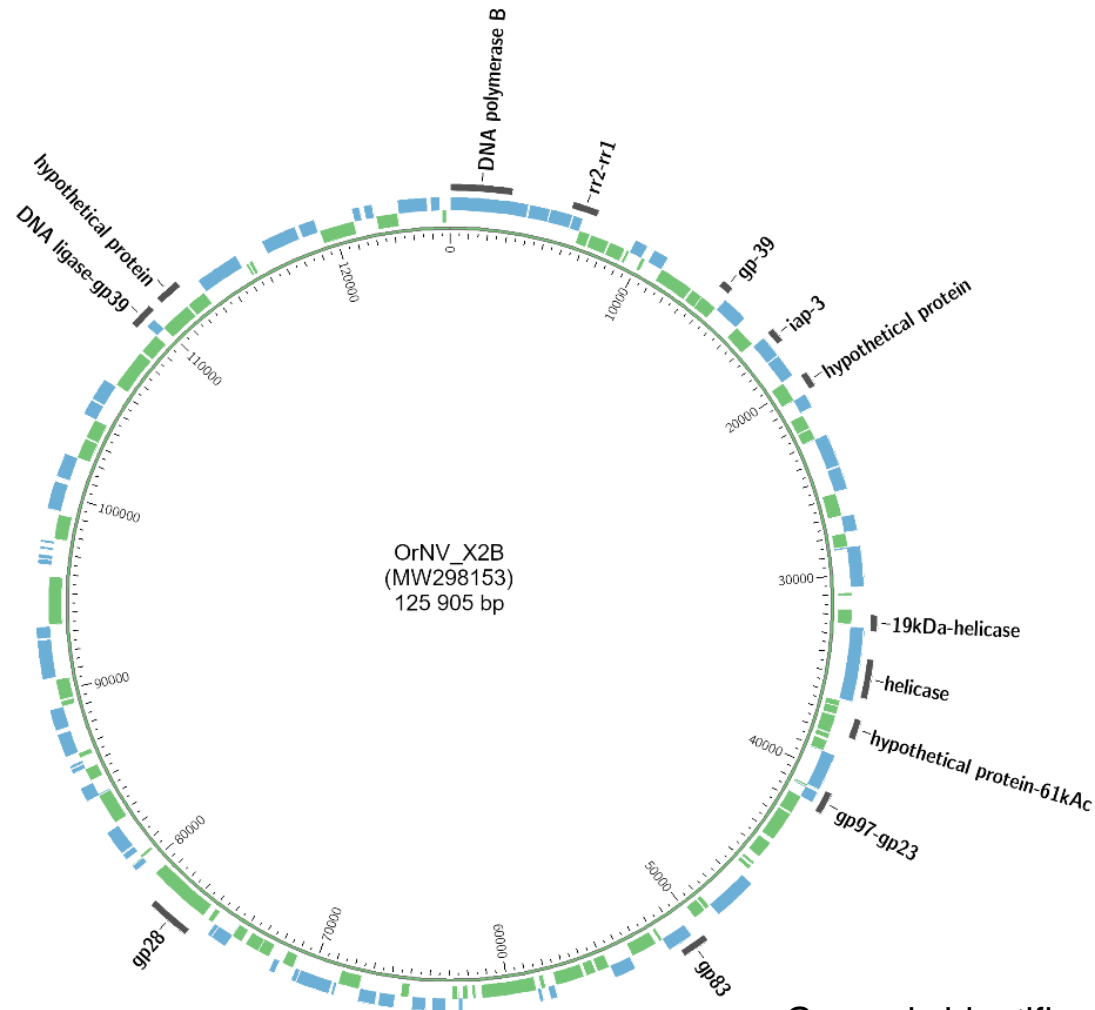
## The CRB project aim

Identify and implement interim and long-term control solutions based on integrated pest management

- Severe damage resulting from lack of effective natural enemies against the new invasions
- Biocontrol agents did not arrive with the invading pest
- New CRB invasions not well controlled by traditionally used biocontrol agents
- CRB management strategies need updating



# OrNV genome

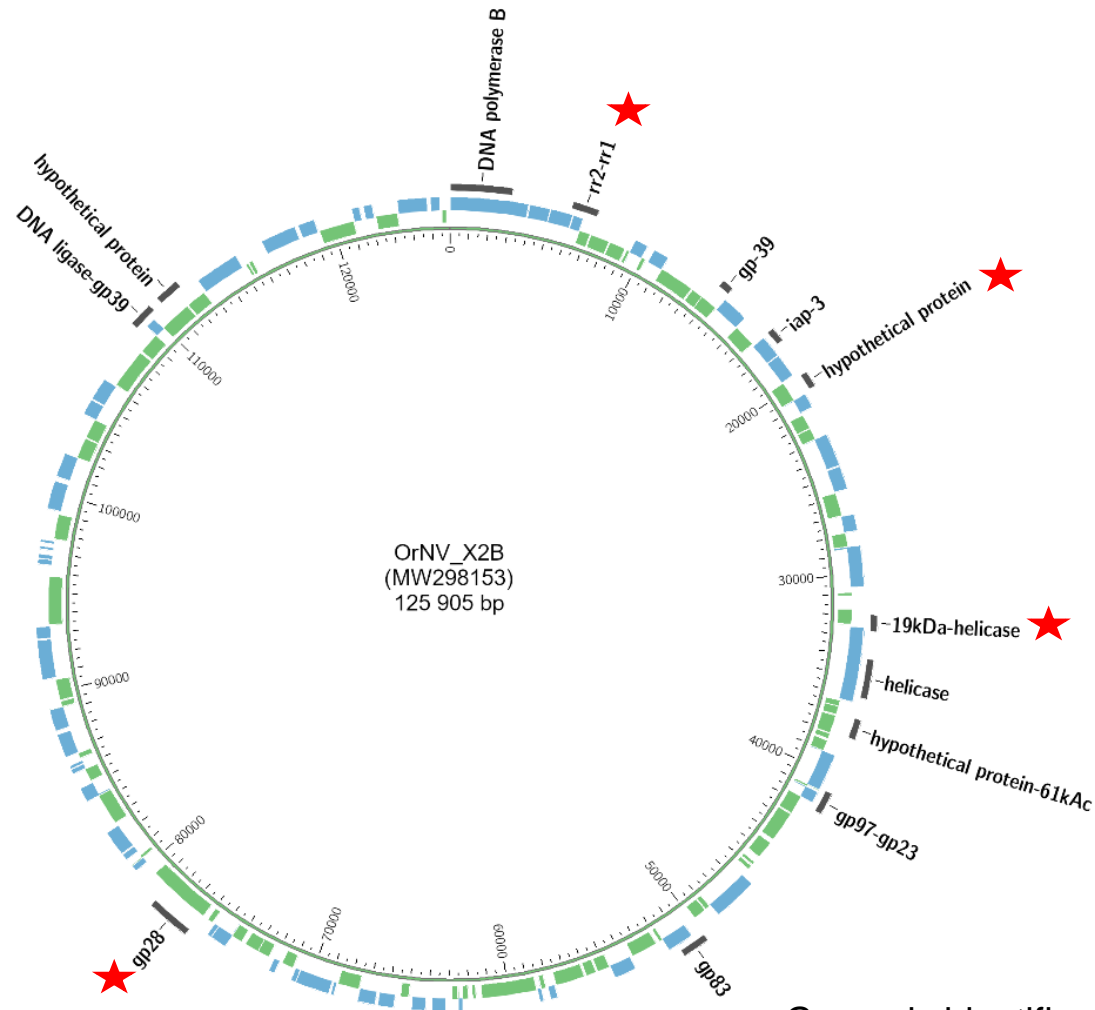


- Genome ~ 125 kb
- dsDNA virus
- DNA markers for 13 OrNV gene regions to differentiate OrNV isolates
- Genomic coordinate locations OrNV isolate X2B (MW298153)

Genomic identification of *Oryctes rhinoceros nudivirus* isolates, a biocontrol agent for coconut rhinoceros beetle.

Ela Hiszczynska-Sawicka *et al.* under review

# OrNV genome



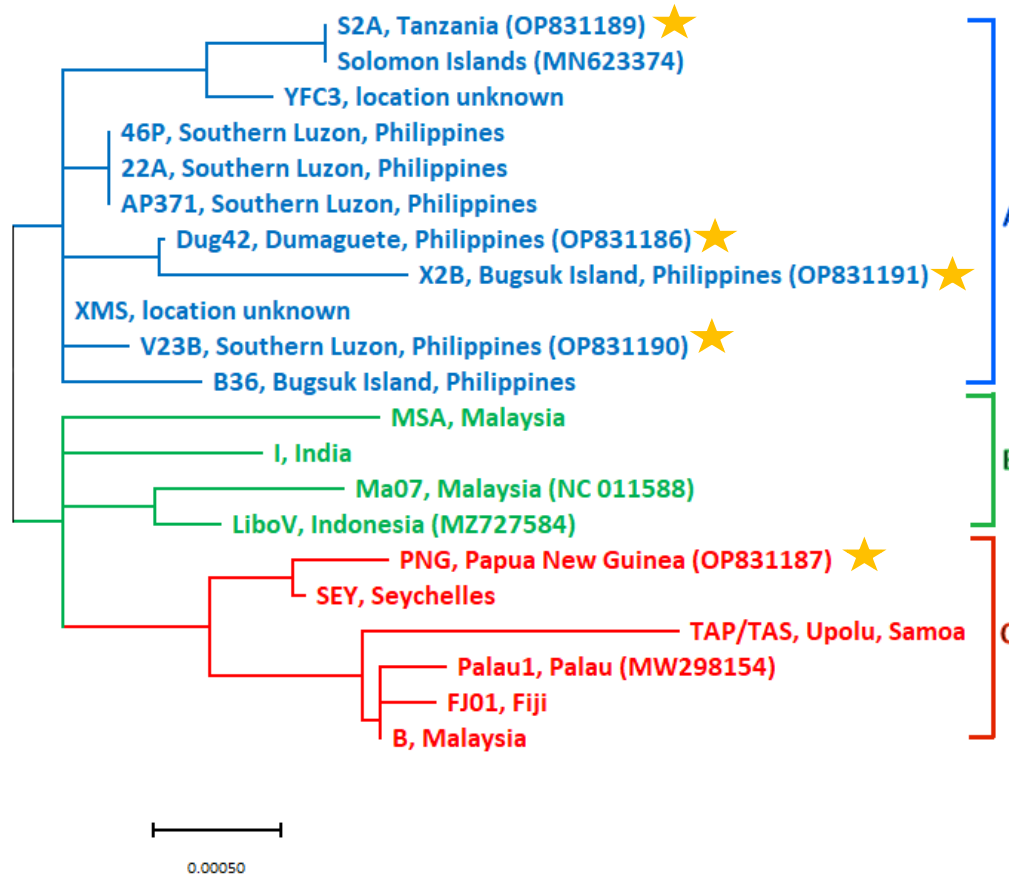
- Genome ~ 125 kb
- dsDNA virus
- DNA markers for 13 OrNV gene regions to differentiate OrNV isolates
- Genomic coordinate locations OrNV isolate X2B (MW298153)
- 4 gene regions for nanopore sequencing

Genomic identification of *Oryctes rhinoceros nudivirus* isolates, a biocontrol agent for coconut rhinoceros beetle.

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# OrNV isolates



- Neighbor-Joining consensus tree
- Concatenated fragments = 3915 bp
- rr2\_rr1, 19 kDa protein, hypothetical protein, gp28\_fragment 1
- 21 OrNV isolates; 17 AgR + 4 public
- Scale bar represents the number of base substitutions per site

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# Genomes of OrNV isolates

Nanopore sequencing of 6 purified virus isolates  
MinION MK1B, flow cells FLO-MIN106 (R9.4.1)

OrNV isolates PV505, V23B, X2B, and PNG combined barcoded library (N50 1,750 bp)  
OrNV-S2A adaptive sequencing (N50 692 bp),  
OrNV-DUG42 (N50 2,150 bp)

Single contig (~30-40X)

**TABLE 1** Sequencing results of six OrNV isolates

OrNV isolate	Isolation location	Yr isolated	GenBank accession no.	SRA accession no.	Genome size (bp)	No. of predicted genes	No. of high-quality reads	Avg coverage (×)
OrNV-PV505	Philippines	1977	<a href="#">OP831188</a>	<a href="#">SRR21977632</a>	125,879	115	3,272	31.7
OrNV-V23B	Philippines	1980	<a href="#">OP831190</a>	<a href="#">SRR21977630</a>	123,988	114	3,163	30.7
OrNV-X2B	Philippines	1983	<a href="#">OP831191</a>	<a href="#">SRR21977629</a>	125,660	118	2,994	27.9
OrNV-PNG	Papua New Guinea	1988	<a href="#">OP831187</a>	<a href="#">SRR21977633</a>	124,855	116	3,503	34.0
OrNV-S2A	Tanzania	1981	<a href="#">OP831189</a>	<a href="#">SRR21977631</a>	125,830	140	3,524	41.3
OrNV-DUG42	Philippines	2016	<a href="#">OP831186</a>	<a href="#">SRR21977634</a>	125,840	114	3,496	41.3

Complete Genome Sequences of Six Isolates of the *Oryctes rhinoceros* Nudivirus  
Mitchell Weston *et al.* doi: 10.1128/mra.00126-23

# Identification of OrNV isolates

Gut samples of CRB for DNA extraction:  
Multiplex PCR      -presence/absence virus  
                             -mtDNA CRB



Virus positive samples:  
-PCR of 7 OrNV genes → barcode amplicons from each sample

-Pool barcoded samples from up to 96 CRB  
-Sequence on MinION MK1B (up to 4 hours)

SNP table to determine OrNV isolate

-Turnaround time (replacing Sanger with ONT)  
-In-house  
-Monitoring environmental CRB samples  
-Testing efficacy of bioassays (lab)  
-Monitoring spread of released biocontrol virus

	Gene region	rr2-rr1 genes region								hypothetical protein (22340 location)										19kDa - helicase				gp28 fragment 1										
	SNP position	5955	5985	6091	6291	6449	6543	6680	7089	19783	19813	19843	19872	19906	19920	19987	20050	20105	20255	20449	31998	32007	32588	32592	32612	32654	76500	76571	76716	76872	76987	77022	77337	77426
	Isolate																																	
1	X2B	T	G	A	C	G	C	G	T	G	T	A	G	C	T	C	G	G	G	G	T	G	G	G	C	C	C	A	C	A	A	G	T	
2	22A	T	G	G	C	G	C	G	T	G	T	A	G	C	T	C	G	G	G	G	C	G	G	G	C	C	C	G	C	G	C	G	T	
3	46P	T	G	G	C	G	C	G	T	G	T	A	G	C	T	C	G	G	G	G	C	G	G	C	C	C	C	G	C	G	C	G	T	
4	AP371	T	G	G	C	G	C	G	T	G	T	A	G	C	T	C	G	G	G	G	C	G	G	C	C	C	C	G	C	G	C	G	T	
5	B	T	G	G	C	G	T	G	C	G	T	A	G	T	C	C	A	G	G	G	C	G	G	C	T	C	C	G	C	G	C	G	T	
6	B36	T	G	G	C	G	C	G	T	G	C	A	G	C	T	A	G	G	G	G	C	G	G	C	C	C	C	C	G	C	G	C	C	
7	Dug42	T	G	G	C	G	C	G	T	G	T	A	G	C	T	C	G	G	G	G	C	G	G	C	C	C	C	C	G	C	G	A	G	T
8	Fiji_Suva	T	G	G	C	G	T	G	C	G	T	A	G	T	C	C	A	G	G	G	C	G	G	A	C	T	C	C	G	C	G	C	G	T
9	I	C	G	G	C	A	C	G	C	G	T	A	G	C	T	C	G	G	G	G	C	A	G	C	C	C	C	C	C	G	C	G	C	T
10	MSA	T	G	G	T	G	C	C	C	G	T	A	G	C	T	C	G	G	G	A	C	G	A	C	C	T	C	G	C	G	C	A	T	
11	PNG	T	G	G	C	G	T	G	C	G	T	A	A	C	T	C	A	G	G	G	C	G	G	C	T	C	C	C	G	A	G	C	G	T
12	PV505	T	G	G	C	G	T	G	C	G	T	A	G	T	C	C	A	G	G	G	C	G	G	C	T	C	C	C	G	C	G	C	G	T
13	S2A	T	A	G	C	G	C	G	T	G	T	C	G	C	T	C	G	G	G	G	C	G	G	T	C	C	T	G	C	G	C	G	C	
14	SEY	T	G	G	C	G	T	G	C	G	T	A	A	C	T	C	G	G	G	G	C	G	G	C	T	C	C	C	G	C	G	C	G	T
15	TAP	T	G	G	C	G	T	G	C	A	T	A	G	T	C	C	A	T	A	G	C	G	T	G	C	T	C	C	G	C	G	C	G	C
16	TAS	T	G	G	C	G	T	G	C	A	T	A	G	T	C	C	A	T	A	G	C	G	T	G	C	T	C	C	G	C	G	C	G	C
17	V23B	T	G	G	C	G	C	T	G	G	T	A	G	C	T	C	G	G	G	G	C	G	G	C	C	C	C	C	G	C	G	C	G	T
18	XMS	T	G	G	C	G	C	G	T	G	T	A	G	C	T	C	G	G	G	G	C	G	G	C	C	C	C	C	G	C	G	C	G	T
19	YFC3	T	G	A	C	G	C	G	T	G	T	A	G	C	T	C	G	G	G	G	C	G	G	T	C	C	T	G	C	G	C	G	C	C

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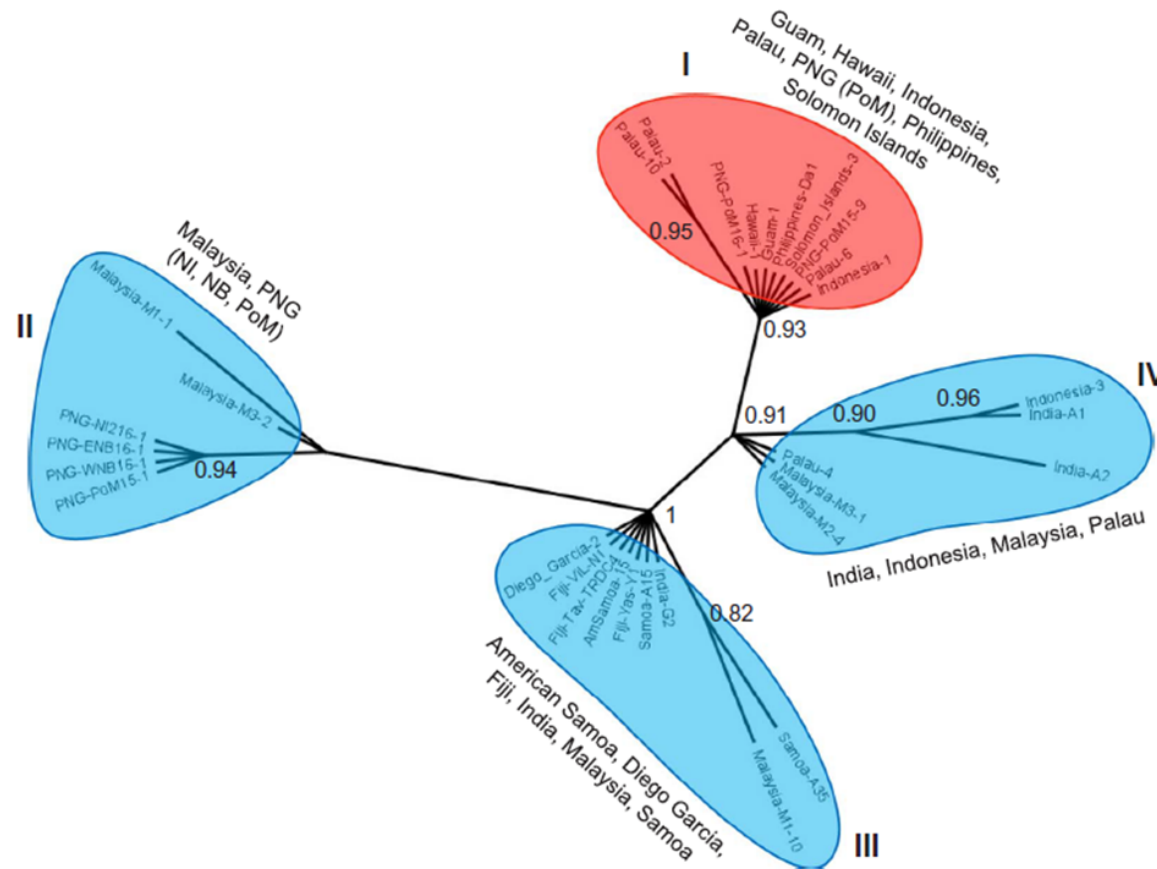
Need to understand the genetic diversity of CRB



# Genetic diversity CRB

S.D.G. Marshall et al.

Journal of Invertebrate Pathology 149 (2017) 127–134



CRB gut samples  
Collection of >7000  
COI mtDNA gene  
Restriction enzyme digest  
Sanger sequence



# Genetic diversity CRB



CRB legs

Collection of >1200 from 12 countries, over 12+ years

Select samples for DNA extraction: 372 samples

‘Clean’ DNA from legs (compared to gut)

Different preservatives (challenge)

Genotyping By Sequencing

# Genotyping By Sequencing (GBS)

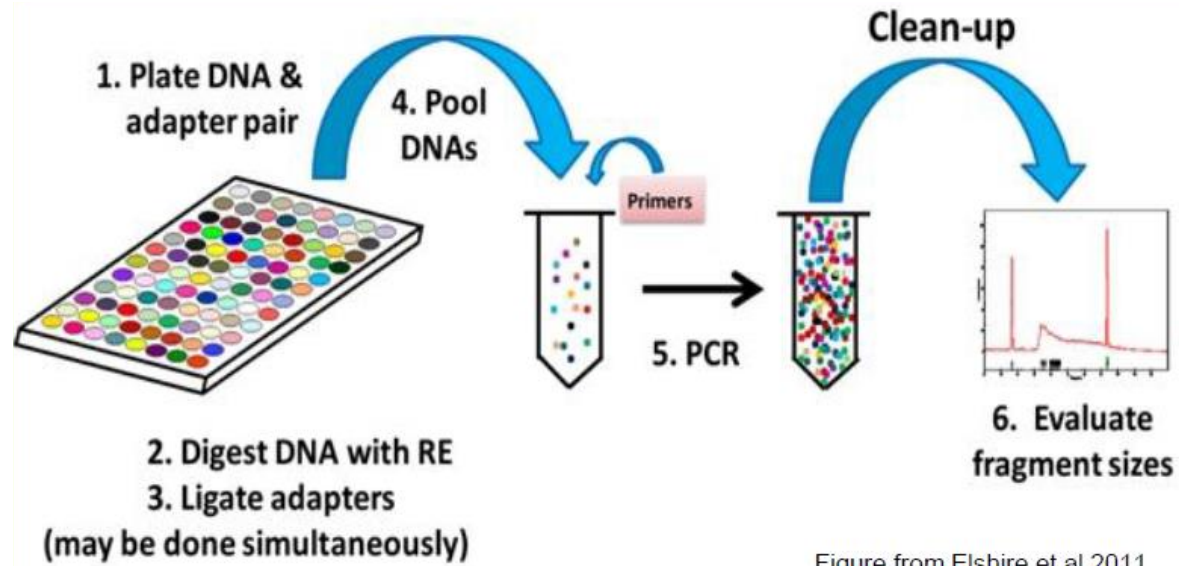


Figure from Elshire et al 2011





KGD = Kinship using GBS with Depth adjustment

Construction of relatedness matrices using genotyping-by-sequencing data

Ken G. Dodds *et al.* BMC Genomics (2015) 16:1047

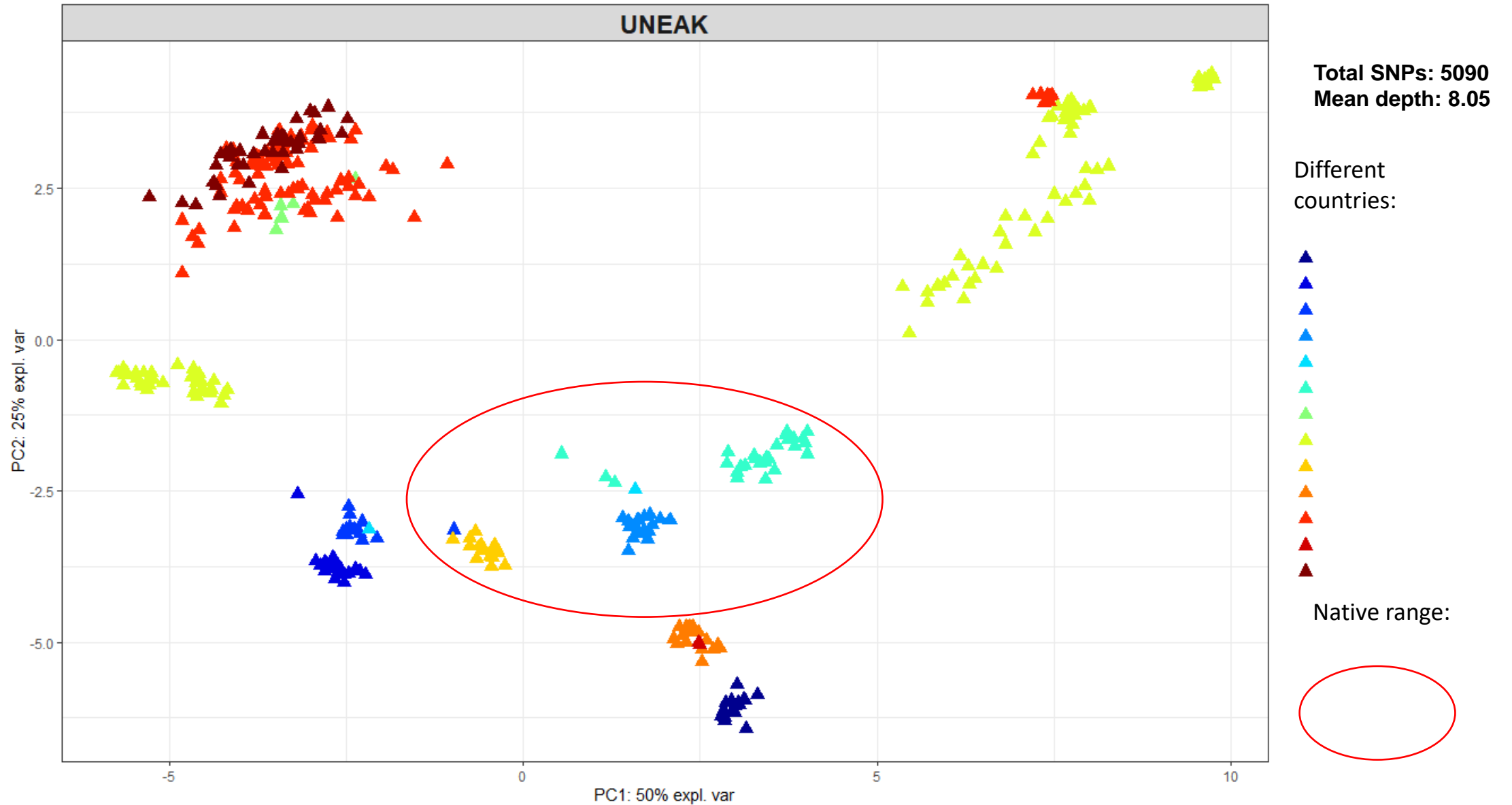
DOI 10.1186/s12864-015-2252-3

<https://github.com/AgResearch/KGD>

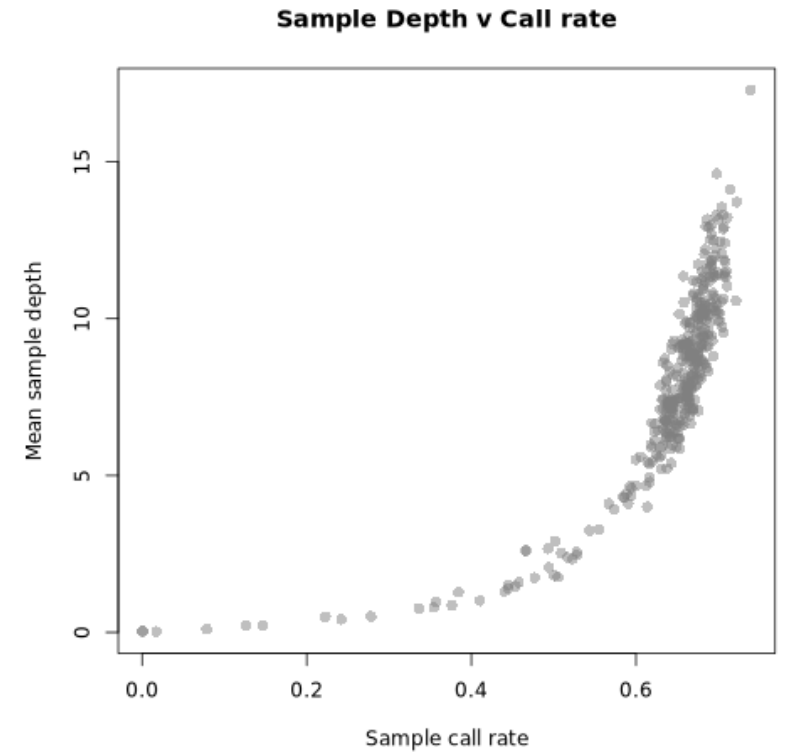
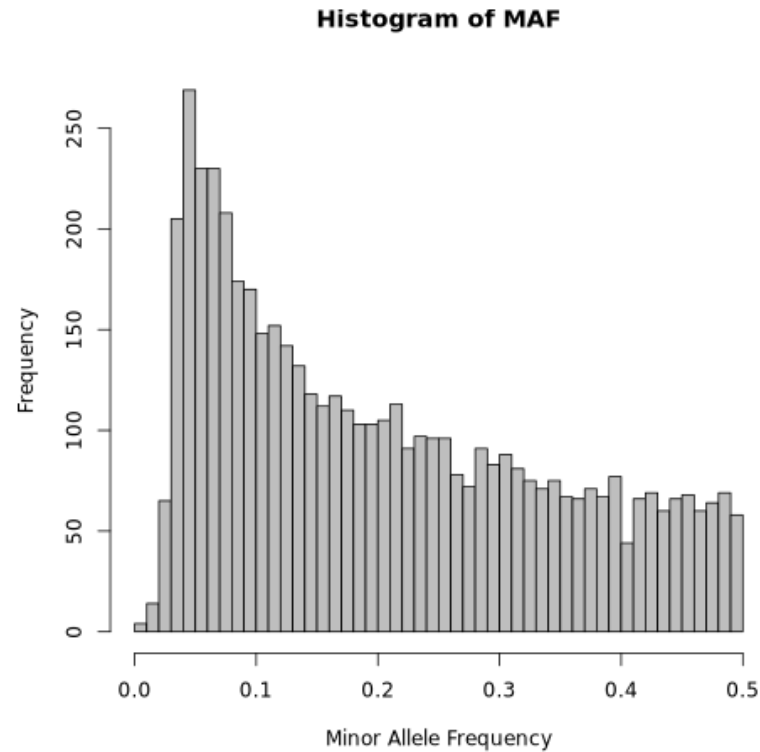
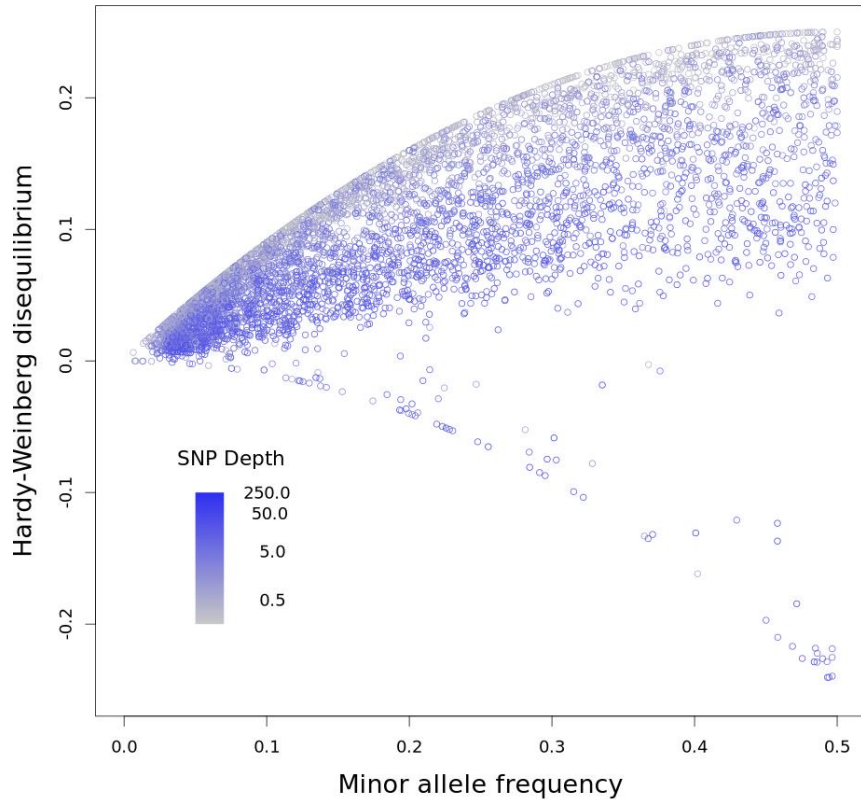
Unbiased relatedness estimation, based on GBS SNPs

Allows low depth GBS genotype calls (including zero depth) to be used

# GBS data analysis; no reference genome

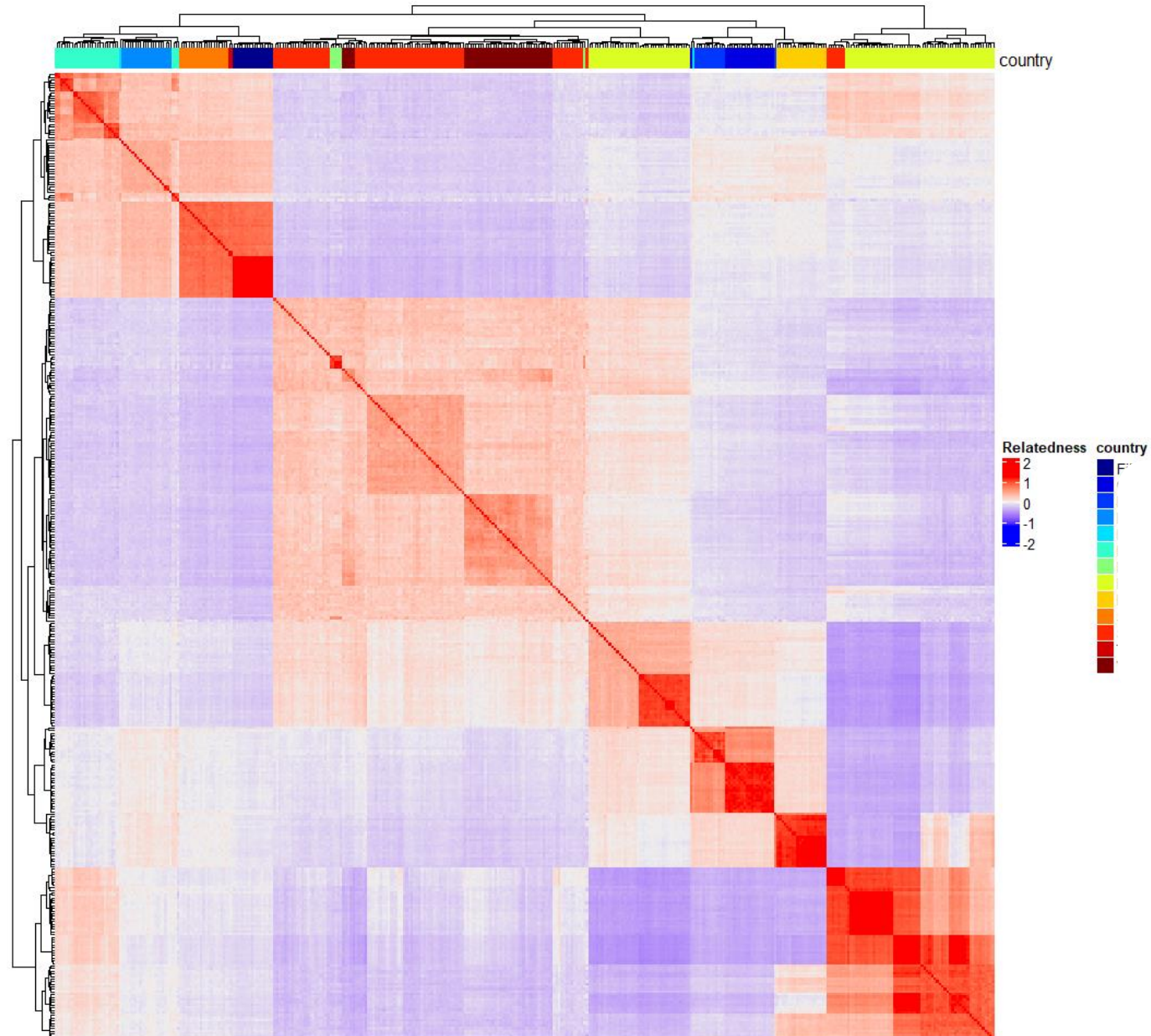


# GBS data analysis; no reference genome





# GBS data analysis; no reference genome



# CRB genome sequence

Assembly	GUAM10	GUAM2G
Sum	366 881 514	375 261 736
N	731	516
Ave	501 889	727 251
Largest	4 934 304	36 074 665
N50	1 073 678	11 120 882

BUSCO	GUAM10	GUAM2G
Complete	98.5%	97.2%
Single	97.7%	97.2%
Duplicate	0.8%	0.7%
Fragment	1.1%	1.1%
Missing	0.4%	1.0%

Additional low depth sequencing of 4 CRB:

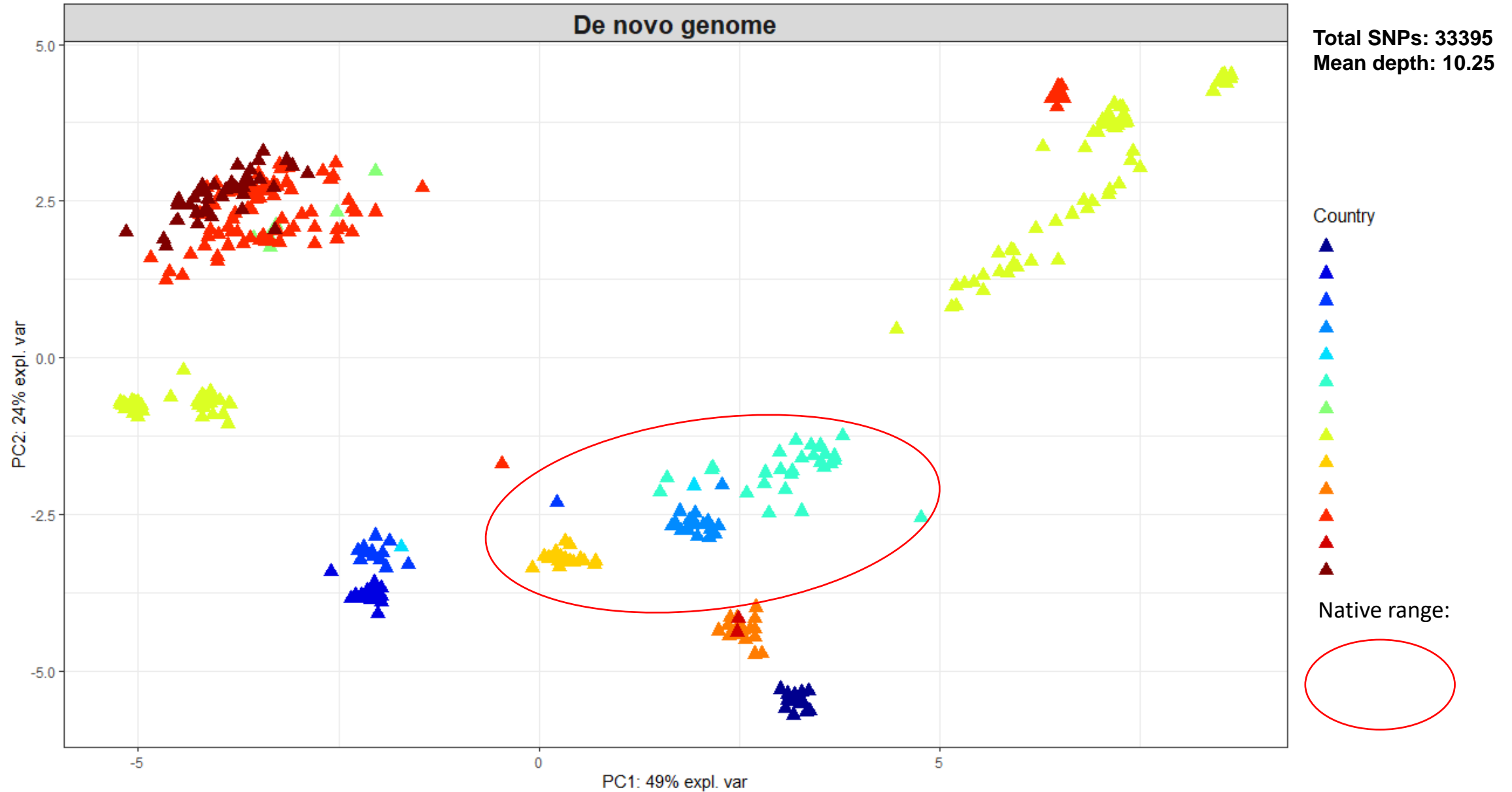
VAN3S	Vanuatu
GUAM4G	Guam
SI-G	Solomon Islands
SI-S	Solomon Islands

S/G cytotype the 'original' COI marker

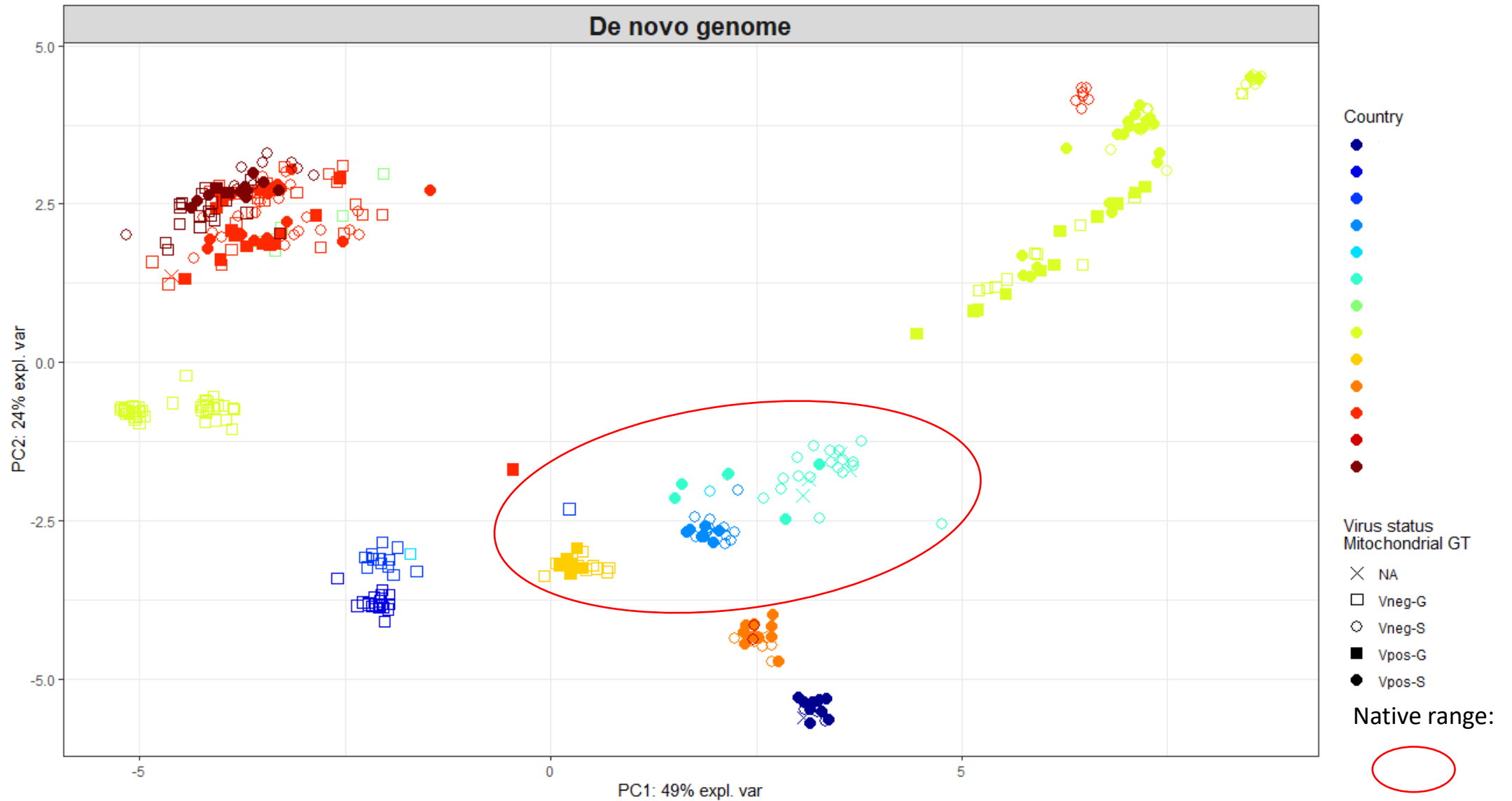
Until recently- no rearing facility for CRB

GenBank:  
377.4Mb, # contigs 1020, N50 10.5Mb

# GBS data analysis; reference genome



# GBS data analysis; reference genome



# GBS data analysis; reference genome

