Development of molecular diagnostics for sugarcane downy mildew

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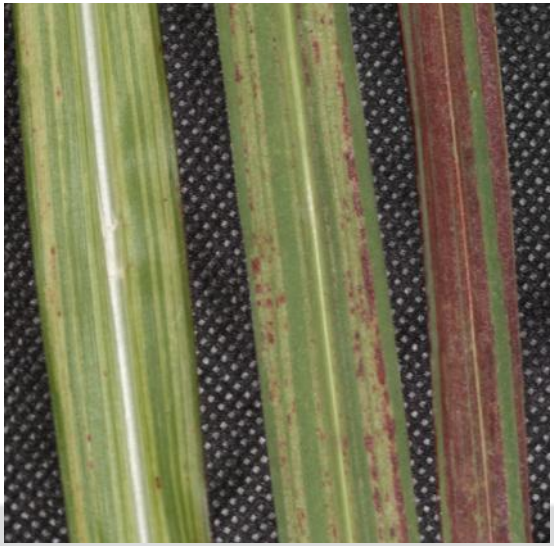
[1] BSES Limited, Australia | [2] Ramu Agri-Industries Limited, Papua New Guinea

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Outline of presentation

- Introduction
  - Downy mildew
  - History of DM in Australia
- Molecular diagnostic design
- Results and conclusions
- Future work





Introduction



- Found in many sugarcane growing regions of the world (SE Asia)
- Downy mildew
  - Caused by Oomycete pathogen in genus *Peronosclerospora*
    - *P. sacchari*, *P. philippinensis*, (others?)
    - Classical taxonomy difficult

Downy mildew symptoms



- Classic leaf stripe
  - White or red in colour
  - Down is visible on underside of leaf in morning



- Leaf shredding
  - May/may not be accompanied with classic symptoms





History lesson

- Downy mildew in Australia in 1910's
- Eradicated from commercial cane in late 1950's by intervention and use of resistant varieties
- BSES ceased resistance screening in 1980's
- Since that time …
  - No selection for DM resistant varieties = reduction of number of DM resistant canes
  - Estimated 60% - 80% of the crop now has susceptibility

Biosecurity project

- SRDC funded project in collaboration with Ramu Agri-Industries Limited, PNG
- Primary aim: screen Australian varieties for resistance to downy mildew, Ramu stunt and *Sesamia* borer.
- Project also allows for some basic research into sugarcane downy mildew
  - Surveys
  - Molecular diagnostics

BSES
Sugarcane for the future

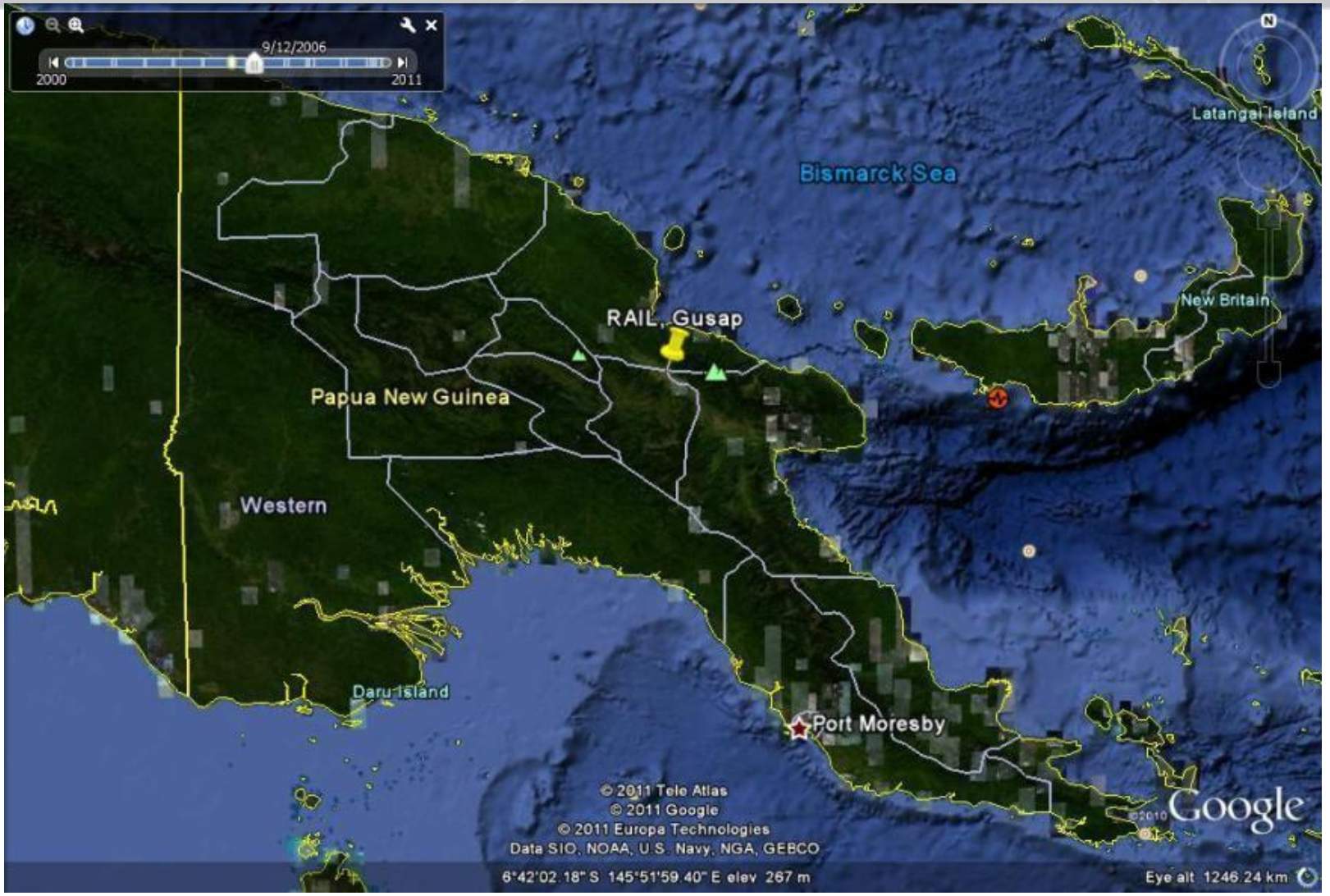
Geography lesson



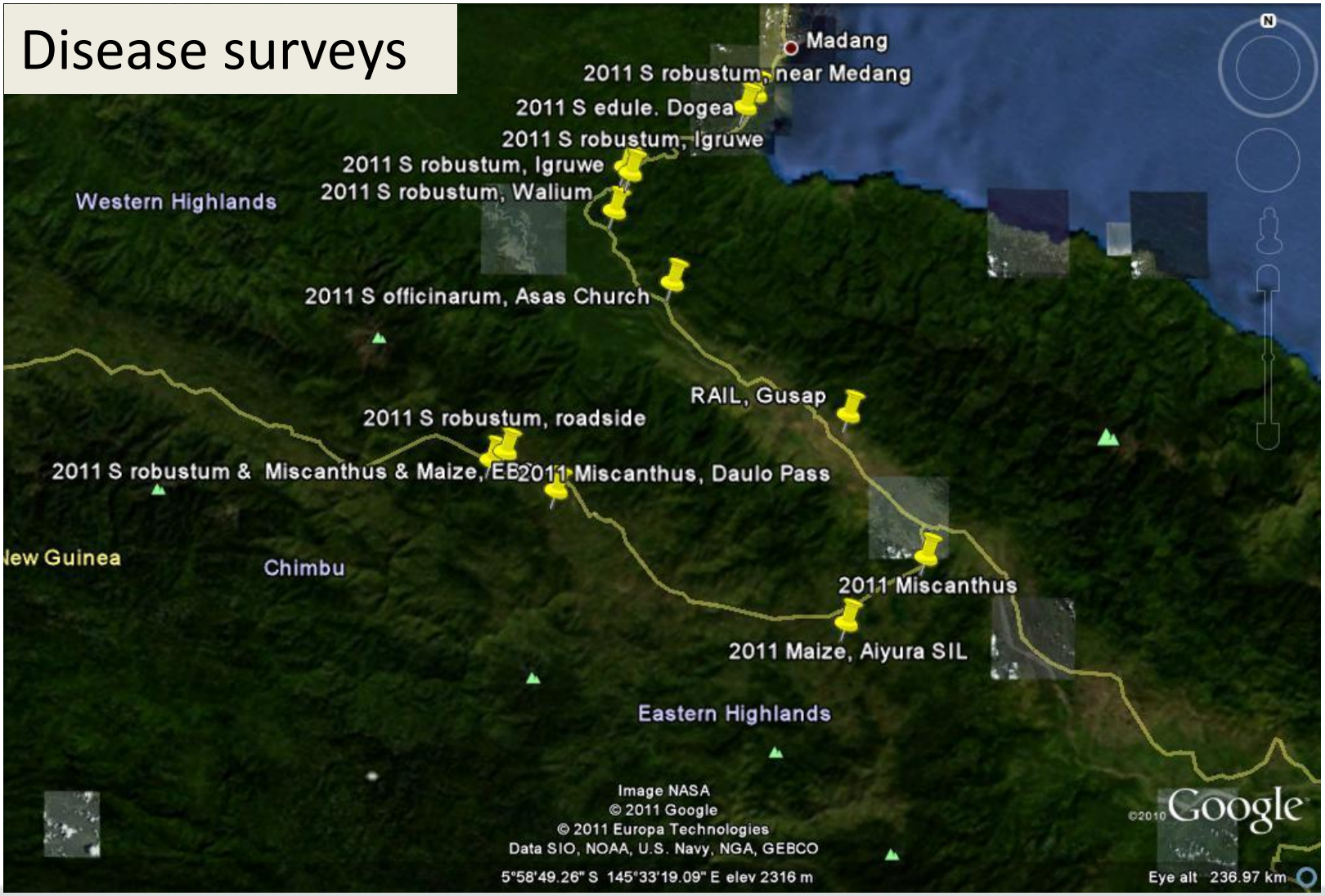



PAPUA NEW GUINEA
LOW
HILLY - MTS
©GraphicMaps.com
Pacific Ocean
Papua New Guinea
worldatlas.com
EQUATOR
0°
Mussau I.
Bismark Archipelago
Lorengau
Admiralty Is.
Kavieng
Vanimo
Wewak
New Ireland
Bismarck Sea
Rabaul
Bougainville Island
New Britain
INDONESIA
Mount Wilhelm
Central Range
Madang
Goroka
Kieta
Lae
Mount Hagen
Huon Gulf
Solomon Sea
Kerema
Kiriwina Is.
D'Entrecasteaux Is.
Solomon Islands
Danu
Gulf of Papua
Torres Strait
Port Moresby
Samarai
worldatlas
Louisiade Archipelago
Coral Sea
Cape York
250 mi
250 km
AUSTRALIA


Papua New Guinea
Gulf of Papua
New Guinea
Wasua
Kiwai
Samari
Sui
Parama
Daru
Jarai
Bula
Aubusi
Bolgu
Kauamag
Saibai
Dauan
Deliverance
Turnagain
Ugar
Torres Strait
Erub
Gabba
Masig
Iama
Poruma
Mer
Mabuiag
Arafura Sea
Badu
Moa
Long
Warraber
Nagir
Waiben
Kirir
Ngurapai
Goode
Friday
Little Adolphus
Mount Adolphus
Muralug
Possession
Turtle Head
Crab
Gulf of Carpentaria
Cape York Peninsula
Coral Sea
Australia
km
0 10 20 30 40 50 60 70 80 90 100


9/12/2006
2000
2011
Latangai Island
Bismarck Sea
New Britain
RAIL, Gusap
Papua New Guinea
Western
Daru Island
Port Moresby
© 2011 Tele Atlas
© 2011 Google
© 2011 Europa Technologies
Data SIO, NOAA, U.S. Navy, NGA, GEBCO
6°42'02.18" S 145°51'59.40" E elev 267 m
Eye alt 1246.24 km
Google

Disease surveys



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Sugarcane for the future













Molecular diagnostic test

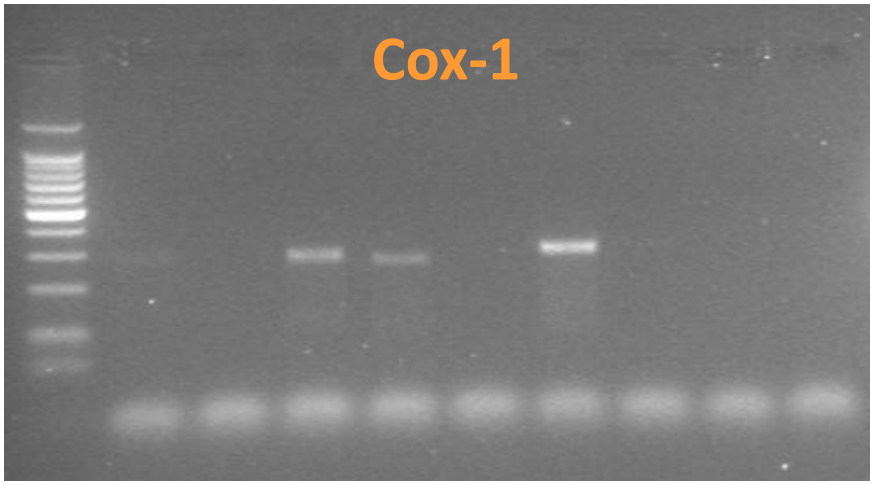
- 2009 visited USA labs to discuss and work on downy mildew (USDA and Texas A&M)
- Gained valuable information including unpublished DNA sequence
- Designed several sets of primers to show differences between *Peronosclerospora* species
  - 4 genome regions targeted

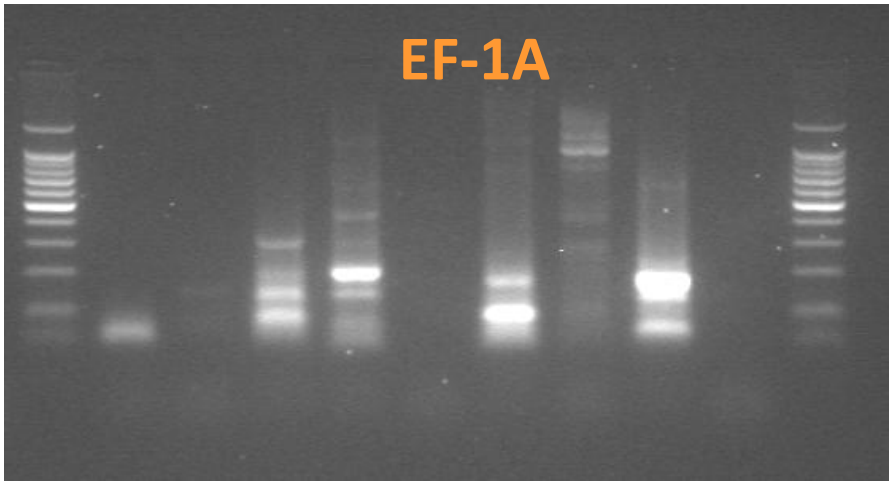
Molecular diagnostic test

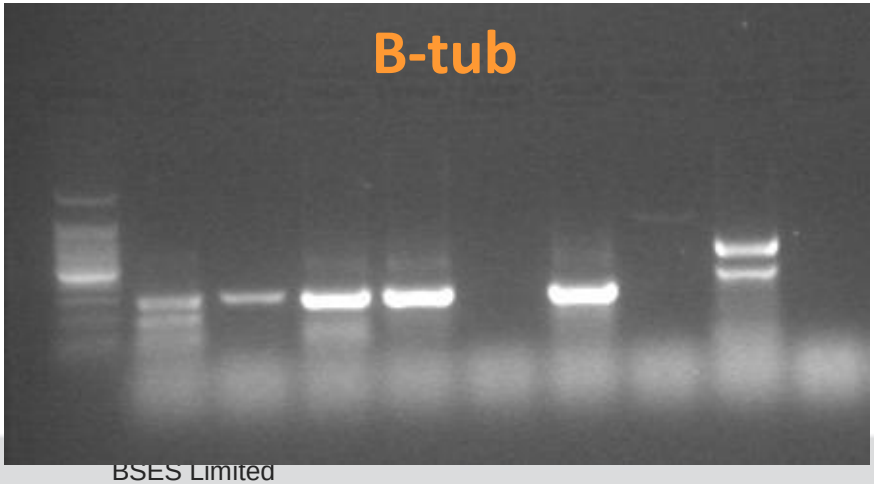
- Designed several sets of primers that could show differences between *Peronosclerospora* species

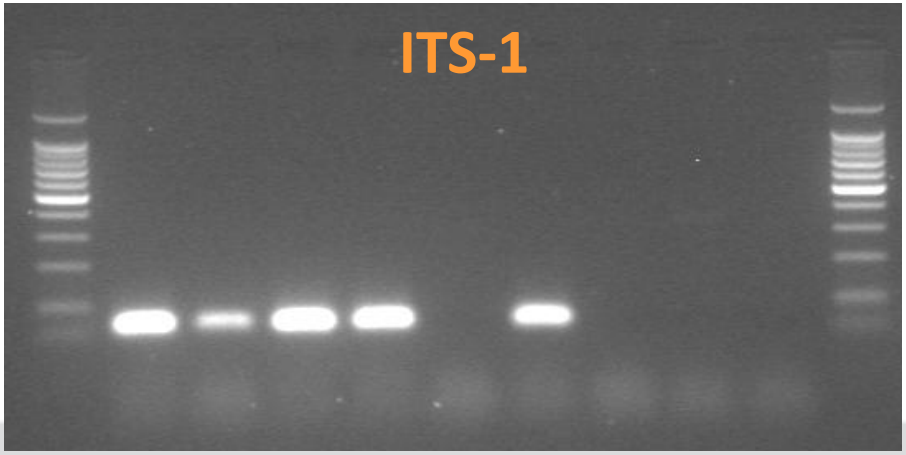
Species	Cox-1	EF-1a	B-tub	ITS-1
P sacchari*	185/295	470?	?	100
P philippinensis*	?	420?	?	100
P maydis	?	410?	400?	100
P sorghi	317	?	400?	100

Primer test/optimisation



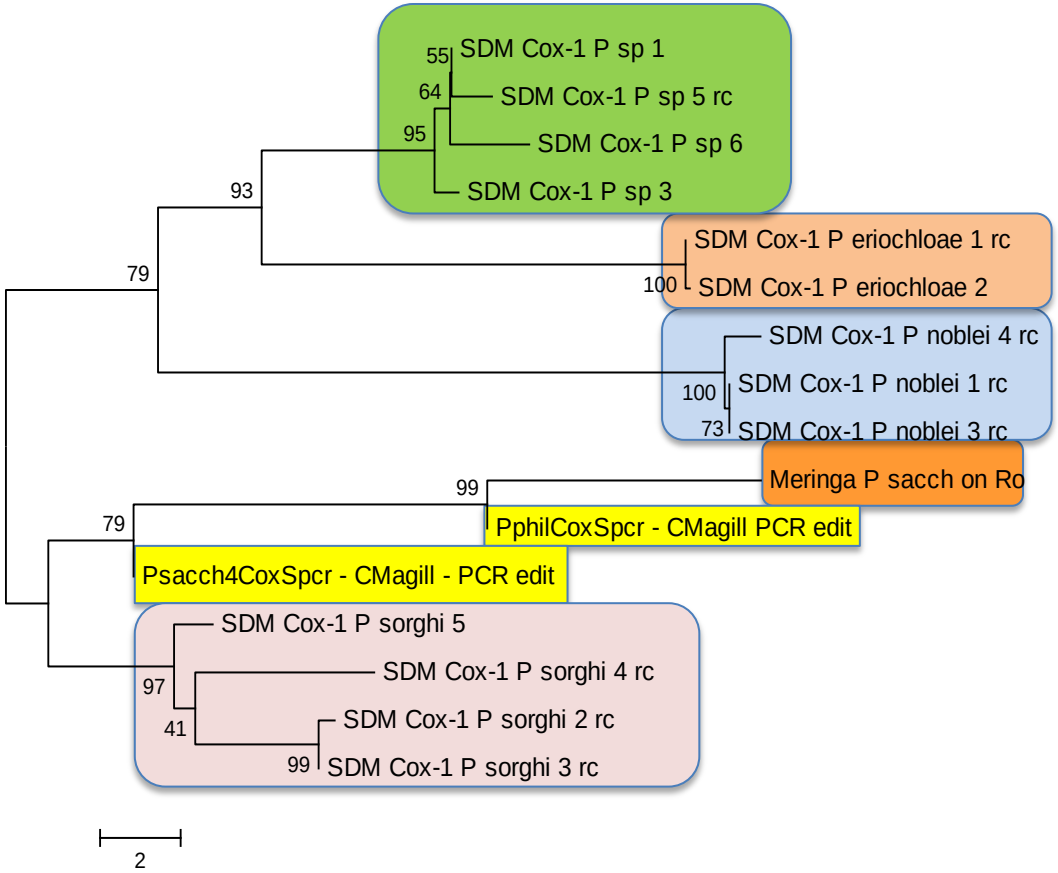






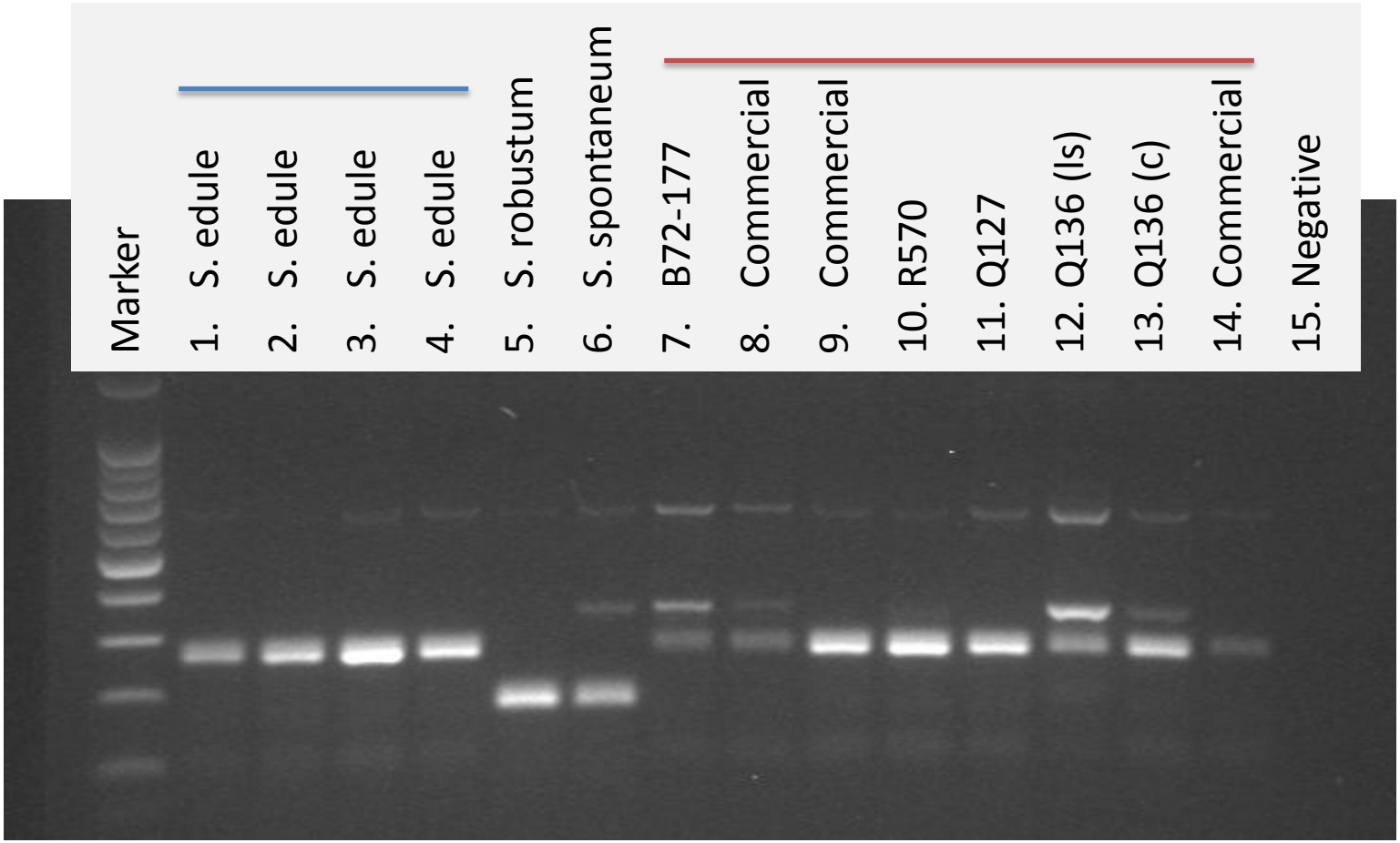
Primers tested on known *Peronosclerospora*

- P. species of same type grouped together
- Proof of concept for Cox-1 primer pair

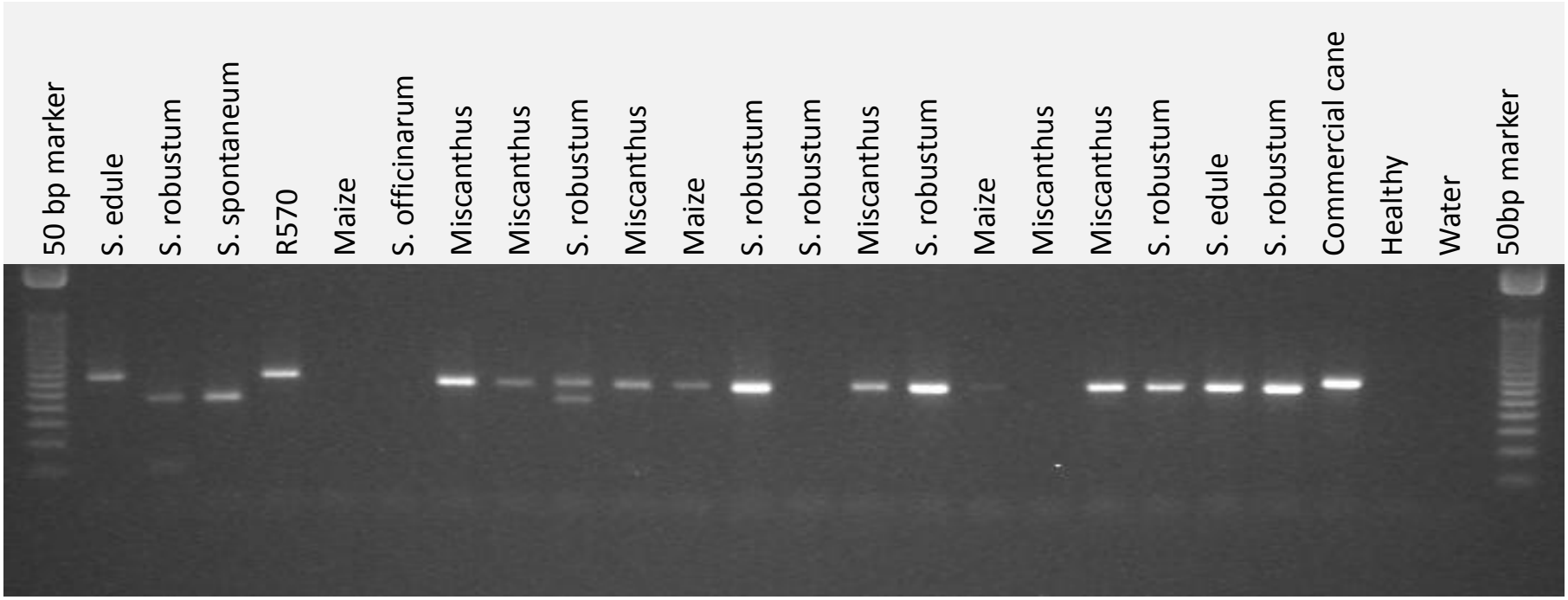


Neighbour joining, 1000 bootstraps (Mega 5)

Cox-1 PCR: first PNG samples

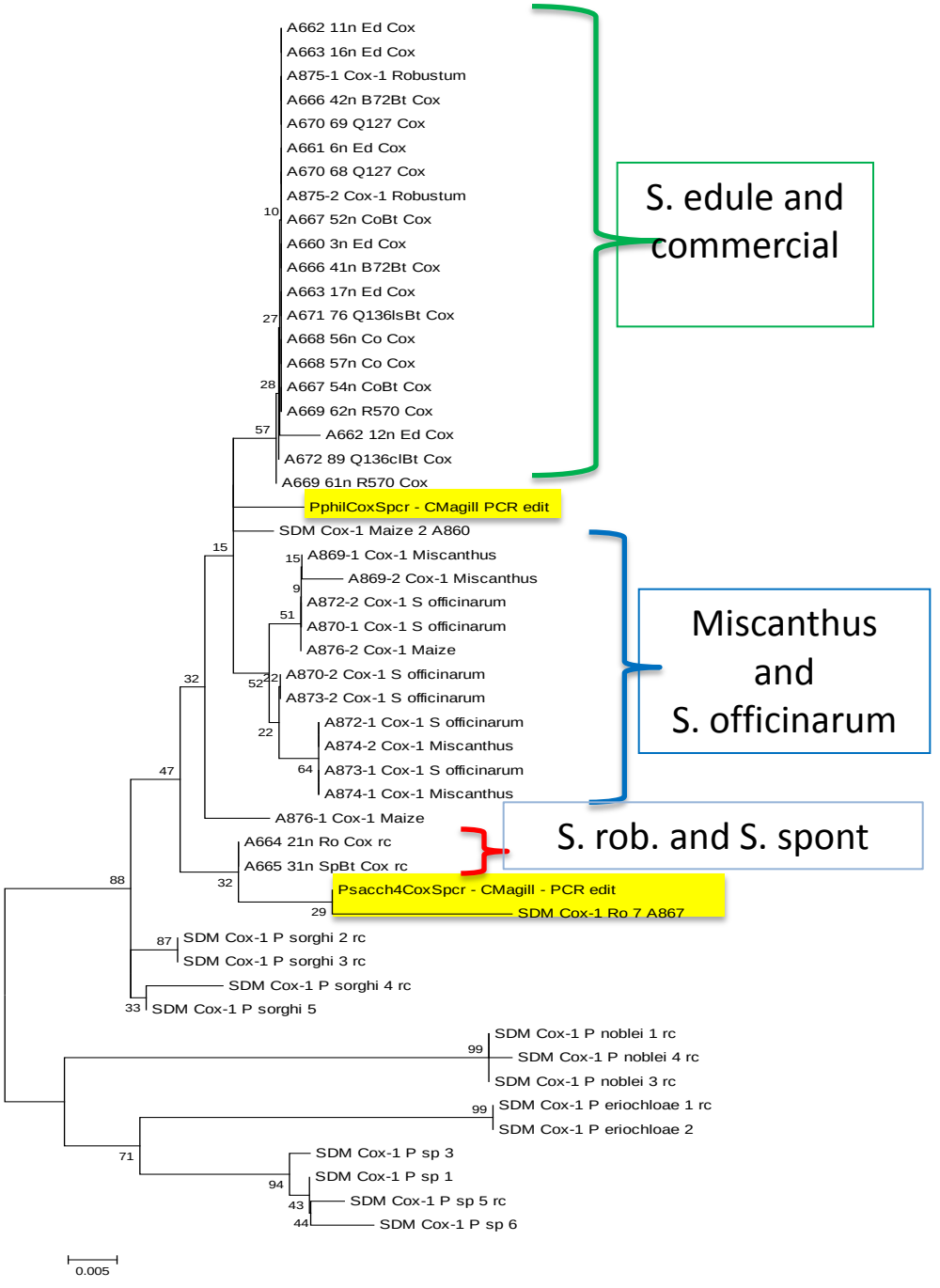


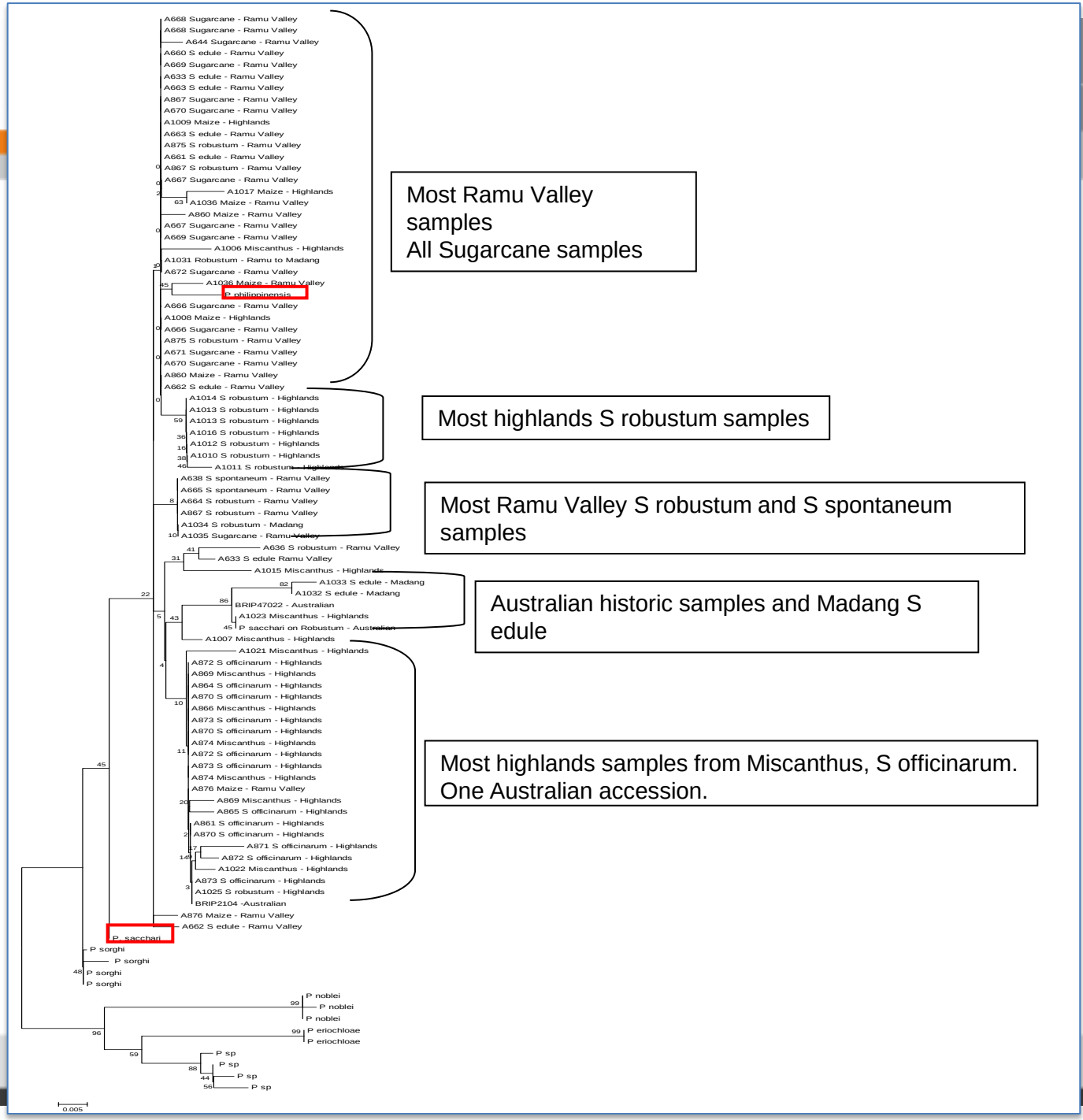
Cox-1 analysis of survey samples



Sequencing of Cox-1 amplicons

3 variants of Cox-1 that are different from previously characterised sequences




A668 Sugarcane - Ramu Valley
A668 Sugarcane - Ramu Valley
A644 Sugarcane - Ramu Valley
A660 S edule - Ramu Valley
A669 Sugarcane - Ramu Valley
A633 S edule - Ramu Valley
A663 S edule - Ramu Valley
A867 Sugarcane - Ramu Valley
A670 Sugarcane - Ramu Valley
A1009 Maize - Highlands
A663 S edule - Ramu Valley
A875 S robustum - Ramu Valley
A661 S edule - Ramu Valley
A867 S robustum - Ramu Valley
A667 Sugarcane - Ramu Valley
A1017 Maize - Highlands
A1036 Maize - Ramu Valley
A860 Maize - Ramu Valley
A667 Sugarcane - Ramu Valley
A669 Sugarcane - Ramu Valley
A1006 Miscanthus - Highlands
A1031 Robustum - Ramu to Madang
A672 Sugarcane - Ramu Valley
A1036 Maize - Ramu Valley
P philippinensis
A666 Sugarcane - Ramu Valley
A1008 Maize - Highlands
A666 Sugarcane - Ramu Valley
A875 S robustum - Ramu Valley
A671 Sugarcane - Ramu Valley
A670 Sugarcane - Ramu Valley
A860 Maize - Ramu Valley
A662 S edule - Ramu Valley
A1014 S robustum - Highlands
A1013 S robustum - Highlands
A1013 S robustum - Highlands
A1016 S robustum - Highlands
A1012 S robustum - Highlands
A1010 S robustum - Highlands
A1011 S robustum - Highlands
A638 S spontaneum - Ramu Valley
A665 S spontaneum - Ramu Valley
A664 S robustum - Ramu Valley
A867 S robustum - Ramu Valley
A1034 S robustum - Madang
A1035 Sugarcane - Ramu Valley
A636 S robustum - Ramu Valley
A633 S edule Ramu Valley
A1015 Miscanthus - Highlands
A1033 S edule - Madang
A1032 S edule - Madang
BRIP47022 - Australian
A1023 Miscanthus - Highlands
P sacchari on Robustum - Australian
A1007 Miscanthus - Highlands
A1021 Miscanthus - Highlands
A872 S officinarum - Highlands
A869 Miscanthus - Highlands
A864 S officinarum - Highlands
A870 S officinarum - Highlands
A866 Miscanthus - Highlands
A873 S officinarum - Highlands
A870 S officinarum - Highlands
A874 Miscanthus - Highlands
A872 S officinarum - Highlands
A873 S officinarum - Highlands
A874 Miscanthus - Highlands
A876 Maize - Ramu Valley
A869 Miscanthus - Highlands
A865 S officinarum - Highlands
A861 S officinarum - Highlands
A870 S officinarum - Highlands
A871 S officinarum - Highlands
A872 S officinarum - Highlands
A1022 Miscanthus - Highlands
A873 S officinarum - Highlands
A1025 S robustum - Highlands
BRIP2104 -Australian
A876 Maize - Ramu Valley
A662 S edule - Ramu Valley
P. sacchari
P sorghi
P sorghi
P sorghi
P sorghi
P noblei
P noblei
P noblei
P eriochloae
P eriochloae
P sp
P sp
P sp
P sp
0.005
Most Ramu Valley samples
All Sugarcane samples
Most highlands S robustum samples
Most Ramu Valley S robustum and S spontaneum samples
Australian historic samples and Madang S edule
Most highlands samples from Miscanthus, S officinarum.
One Australian accession.

Ramu Agri-Industries plantation results

- Possibly 2 variants of *Peronosclerospora* present in the Ramu Valley
  - Predominantly one Cox-1 variant in the commercial plantation
  - Predominantly one symptom in the commercial plantation (classic symptoms)
    - One variety (Q136) had both symptoms, but only one Cox-1 variant.
  - **Cox-1 variant does not appear to be correlated with symptoms**



Results from survey

- Miscanthus and *S. robustum* leaf shredding is amplified by Cox-1 primers
- Not a lot of classical symptoms observed outside of Ramu Valley
  - Only on *S. officinarum*, *S. edule* and maize
  - Reported on *S. robustum*, but not found unless associated with leaf shredding

DM variation in PNG

- Sequence of Cox-1 revealed loose groupings:
  - *S. robustum* from highlands (highest point)
  - *S. officinarum* and *Miscanthus* from highlands
  - *P. philippinensis* clustered amongst commercial varieties on RAIL estate
  - *P. sacchari* fell outside the clusters
  - DM sample on *S. edule* near Madang is most similar to the herbarium specimen from Qld in 1980's.

Conclusions

- Cox-1 primer pair good for determining differences in DM
  - Species? Need more type specimens
- Variation due to location
  - Secondary due to host species



Future work

- Survey sample analysis and sample collection
- Analysis of key samples from other countries
- Microscopic analysis of *Peronosclerospora* species in PNG





Acknowledgments

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- Biosecurity Queensland (Qld DEEDI)
  - Dr. Roger Shivas (Herbarium)
- Funding from SRDC

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A part of the NBPOL group

Australian Government
Sugar Research and Development Corporation

Call for specimens!

- If you have access to sugarcane downy mildew specimens and are able to send them
  - Especially if you have some known/type specimens
- All costs of shipping covered
- nthompson@bses.com.au



