The origins of crop disease: the curious case of kiwifruit canker

Honour McCann[1,2], Erik H. A. Rikkerink[3], Frederic Bertels[1,4], Mark Fiers[5], Ashley Lu[5], Jonathan Rees-George[3], Mark T. Andersen[3], Andrew P. Gleave[3], Bernhard Haubold[6], Mark W. Wohlers[3], David S. Guttman[2], Pauline W. Wang[2], Christina Straub[1], Joel Vanneste[7], Paul B. Rainey[1,6,*,¶], Matthew D. Templeton[3,8,*,¶]

[1]New Zealand Institute for Advanced Study and Allan Wilson Centre, Massey University, Auckland, New Zealand, [2]Centre for the Analysis of Genome Evolution & Function, University of Toronto, Toronto Canada, [3]The New Zealand Institute for Plant & Food Research Limited, Auckland, New Zealand, [4]Biozentrum, University of Basel and Swiss Institute of Bioinformatics, Basel, Switzerland [5] The New Zealand Institute for Plant & Food Research Limited, Lincoln, New Zealand, [6]Max Planck Institute for Evolutionary Biology, Plön, Germany, The New Zealand Institute for Plant & Food Research Limited, Ruakura, Hamilton, New Zealand, [8]School of Biological Sciences, University of Auckland, Auckland, New Zealand. *Joint senior authors.

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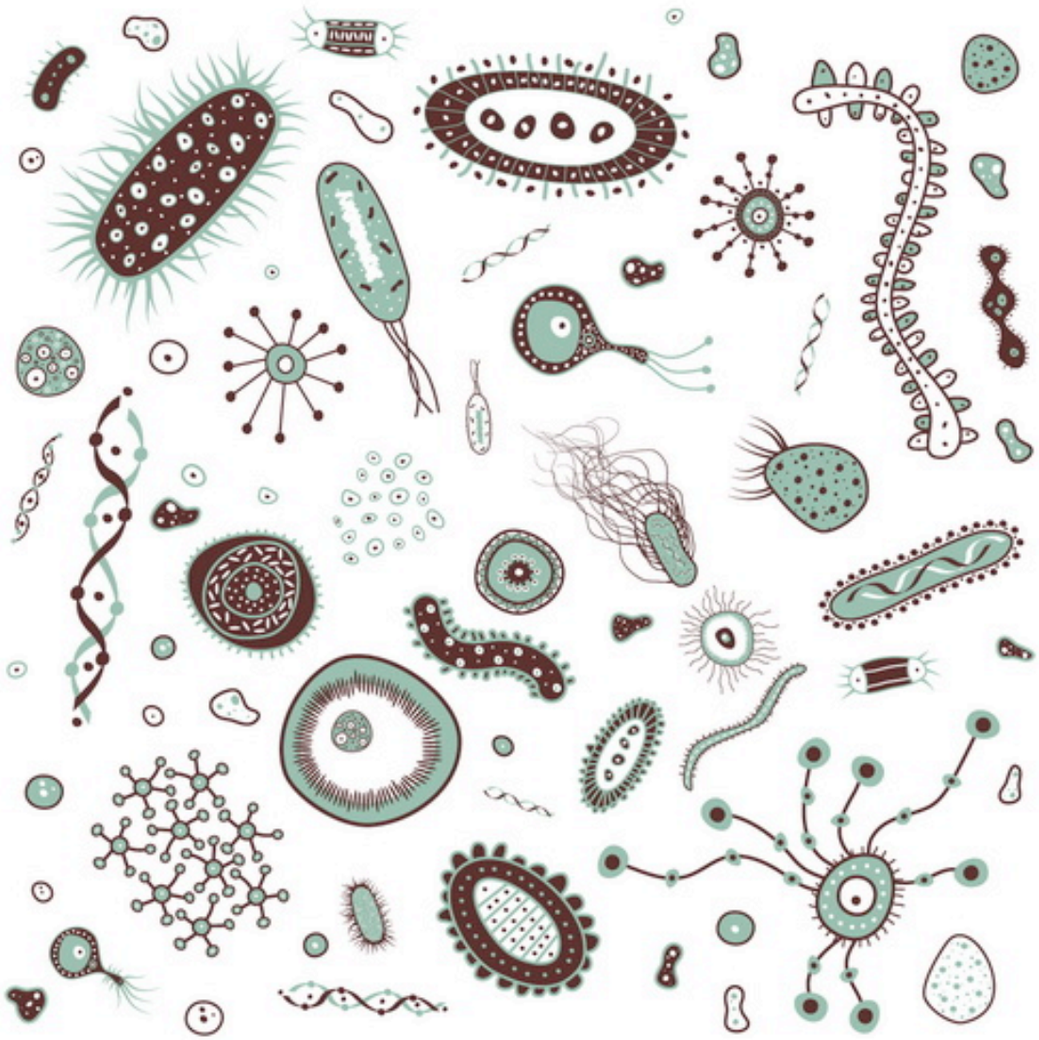
Nature is rare nice

- Microbes need food, plants are food...



Plant pathogens matter

- Significant human cost
- ~20% annual loss to food production
- Irish potato famine (1845-52)
- Wheat stem rust (1999-)
- Psa on kiwifruit (1984-)

Where do pathogens of crop plants come from?

- Zoonoses and human disease...
- Emergence of crop pathogens by sampling from pathogens of wild relatives, vs. host shift
- Domestication likely provides opportunity for the evolution of virulence (e.g., mono-culture, densely planted fields)
- Two questions
  - Where and on what did a given pathogen evolve (evolutionary history)?
    - Source population?
  - What factors underpin outbreaks of disease?
    - Transmission from source vs alternate host, population expansion, dissemination

Kiwifruit - a recently domesticated crop

- ~70 species of *Actinidia* native to China and south east Asia.
- Fruit long collected from wild vines
- Isabel Fraser (1904) brings seeds from China to Whanganui (40 seedlings)
- Hayward Wright (1925) selects one seedling and establishes the green flesh *A. deliciosa* 'Hayward' variety (accounts for 99 % of commercial crop plants)
- Export of Chinese gooseberries begins in 1959
- Links between China and NZ initiated in 1943-44 (Dr Li Lairong) led to further importation of seed (1975) and selection of a second clone *A. chinensis* 'Hort 16A'
- Plant Variety Rights to Hort 16A granted in 1995
- Kiwifruit grown in NZ, Italy, France, Chile, Japan, Korea, USA
- $4.5 billion industry

The inevitability of disease

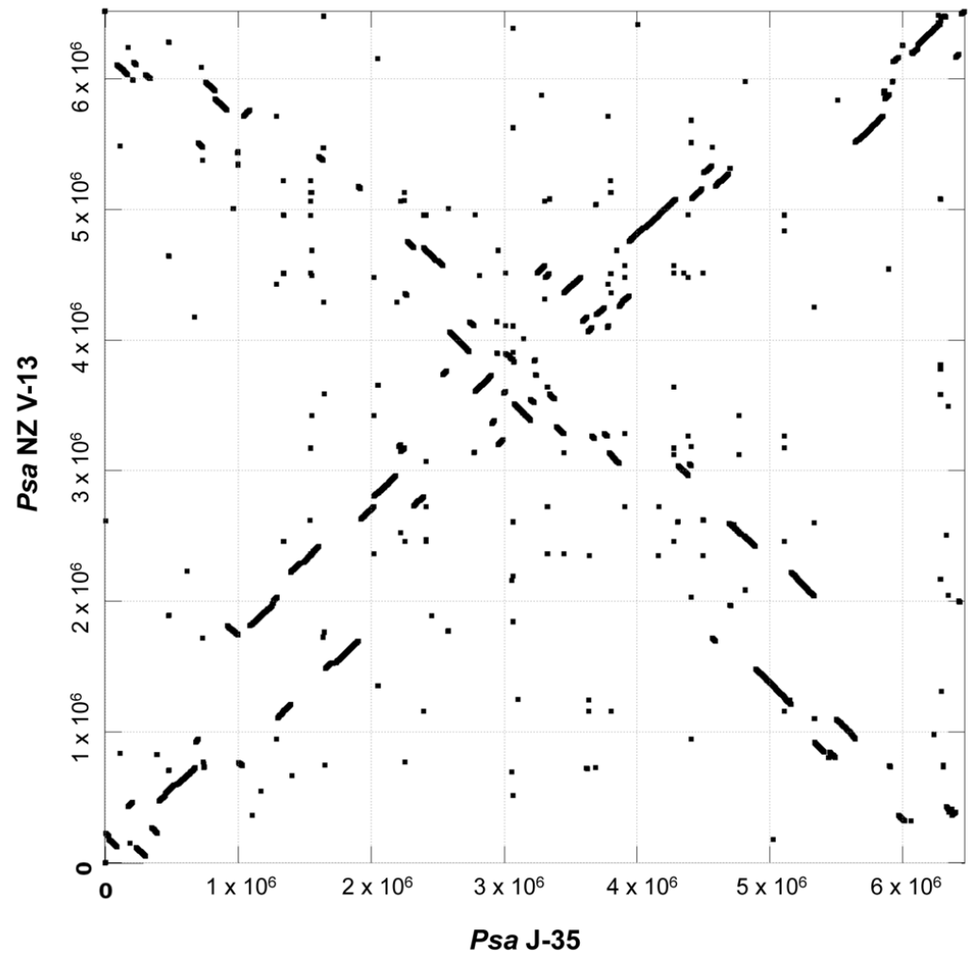
- Disease (fungal and bacterial) noticed from earliest days of commercial plantings
- Kiwifruit canker disease first recorded in Japan (1980) (and Hunan, China); Korea (1988); Italy (1992); Italy (2008); Rapid global transmission of *Psa*-V: Te Puke (2010)

The upside of Kiwifruit canker disease

- Opportunity to understand the emergence of a crop disease concomitant with domestication of its host
- History of domestication known
- History of disease known (and isolates archived)
- Some knowledge of bacterial genotypes
- Draft genomes & three recent *PLoS One* papers
- Industry in apportioning blame (based on minimal SNPs and inferences that disregard recombination)
- Much uncertainty and need for population-level analyses

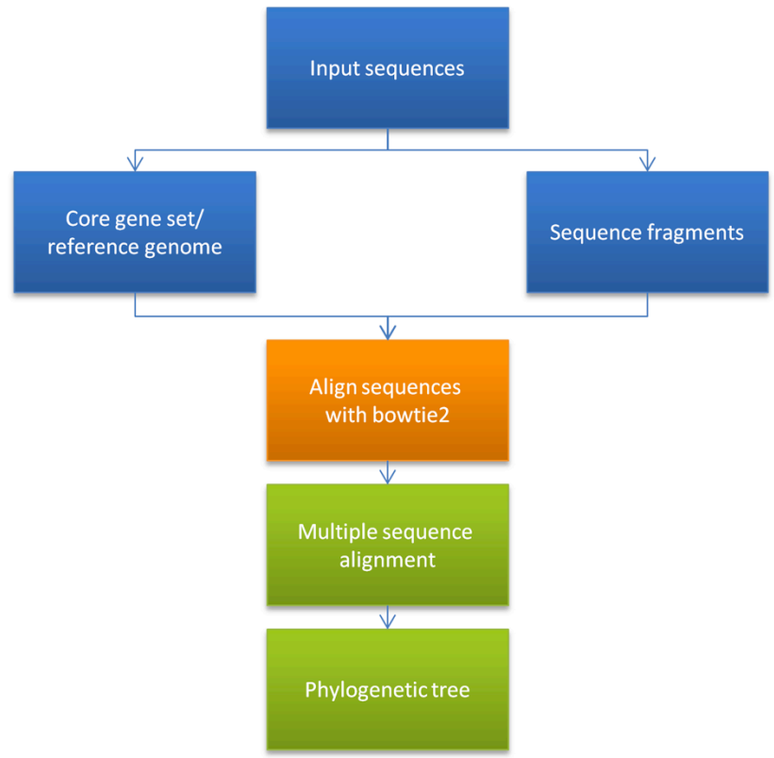
Two complete genomes

- Synteny plot



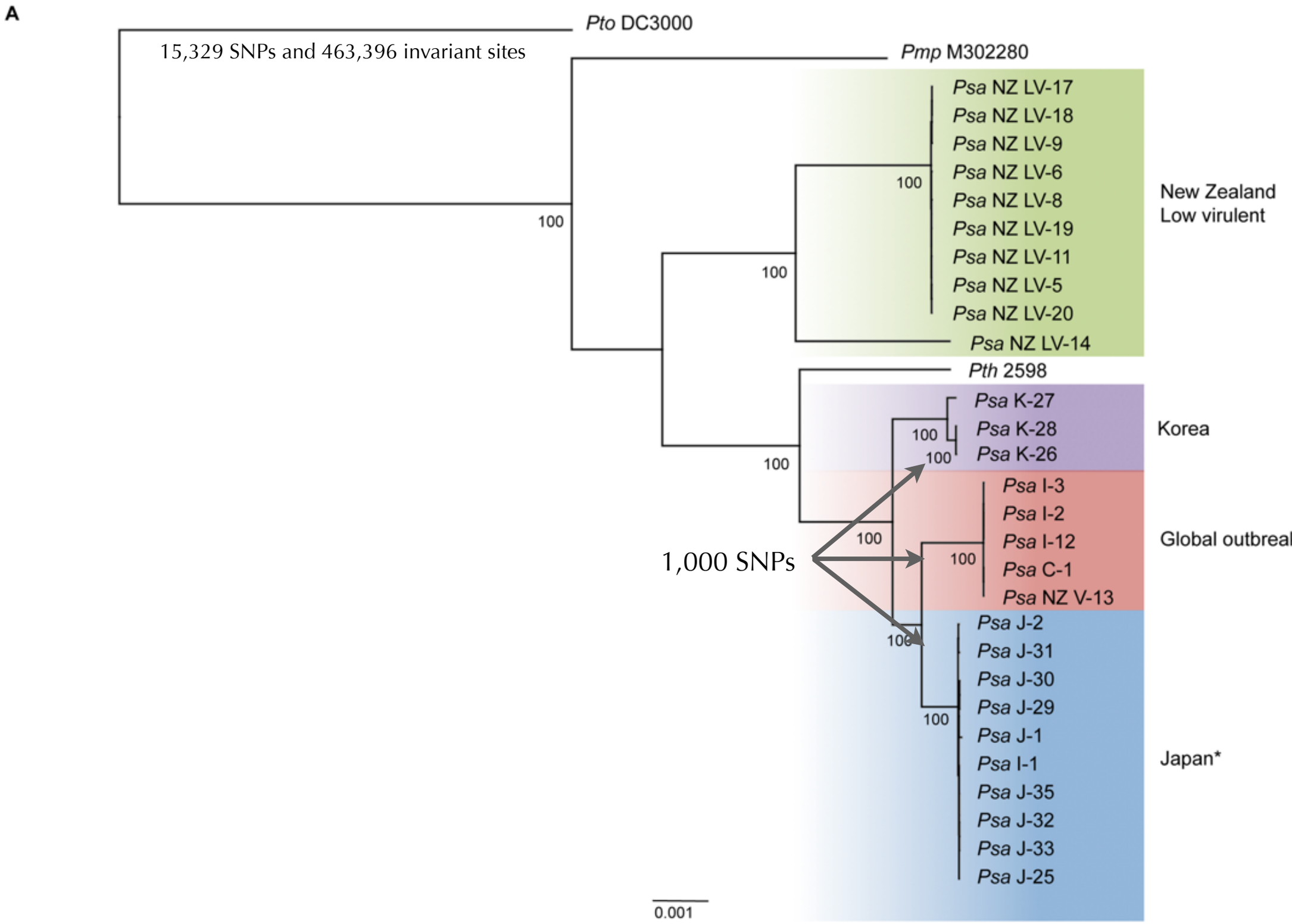
SNP extraction and tree building (REALPHY)

- SNPs (variant and invariant) from any sequence data
- No need for read processing
- Output multiple alignment
- Output trees
- Tune parameters
- Importance of bias due reference
- Importance of using both variant and invariant sites

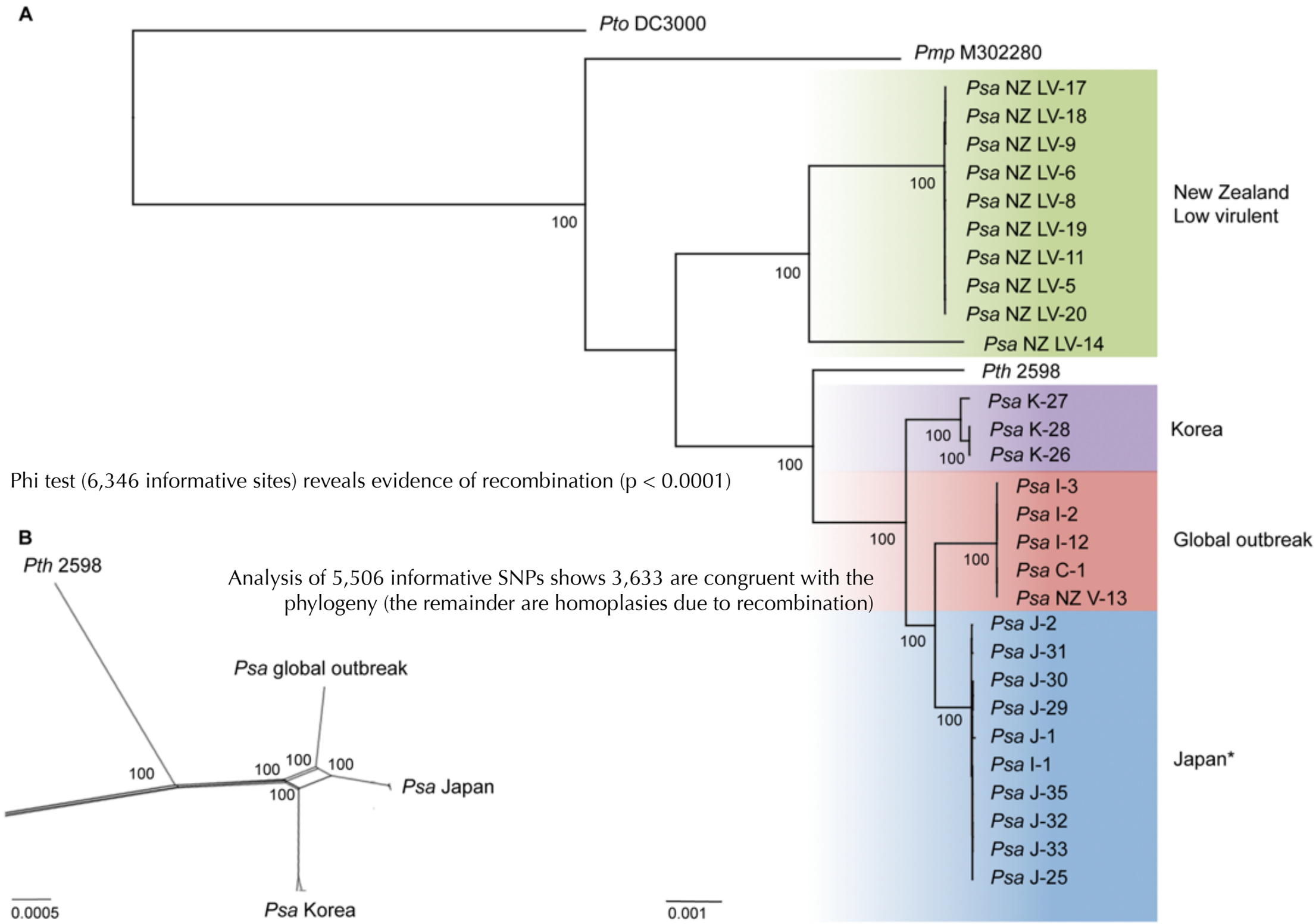


Bertels et al NAR under review

Phylogeny suggests independent samplings from a source population



Recombination supports notion of source population

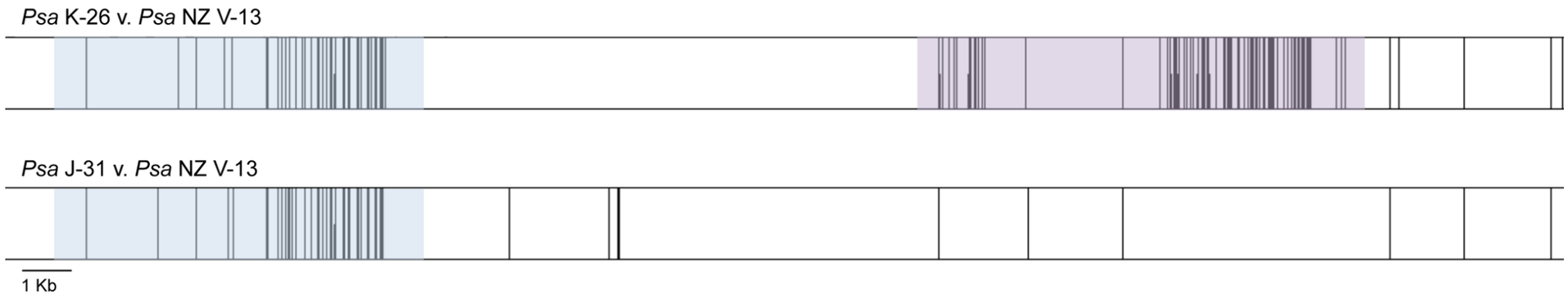


Phi test (6,346 informative sites) reveals evidence of recombination ($p < 0.0001$)

Analysis of 5,506 informative SNPs shows 3,633 are congruent with the phylogeny (the remainder are homoplasies due to recombination)

Linkage disequilibrium ‘blocks’

- Mapping Japanese (J-31) and Korean (K-26) ‘reads’ to NZ V-13



- GENCONV (Sawyers 1989)

Strain pair[1]	Total length[2] (kb)	Regions[3]	Average length[4] (kb)	Proportion of genome[5]	Recombinant SNPs[6]	Proportion of recombinant SNPs
K-26[7]	427.8	172	2.49	0.083	6,085	0.214
J-31[7]	184.4	134	1.38	0.036	2,413	0.085
NZ V-13[7]	302.4	159	1.90	0.059	4,218	0.149
NZ V-13 v. J-31	427.5	173	2.47	0.083	6,073	0.214
K-26 v. J-31	299.8	158	1.90	0.058	4,179	0.147
NZ V-13 v. K-26	183.9	132	1.39	0.036	2,371	0.083

- Putative gene conversion events have low dN/dS (0.088 ± 0.002 vs. 0.13 ± 0.004 (purifying selection)
- Overall the population structure toward the clonal end (SNPs are twice as likely to be generated by mutation)

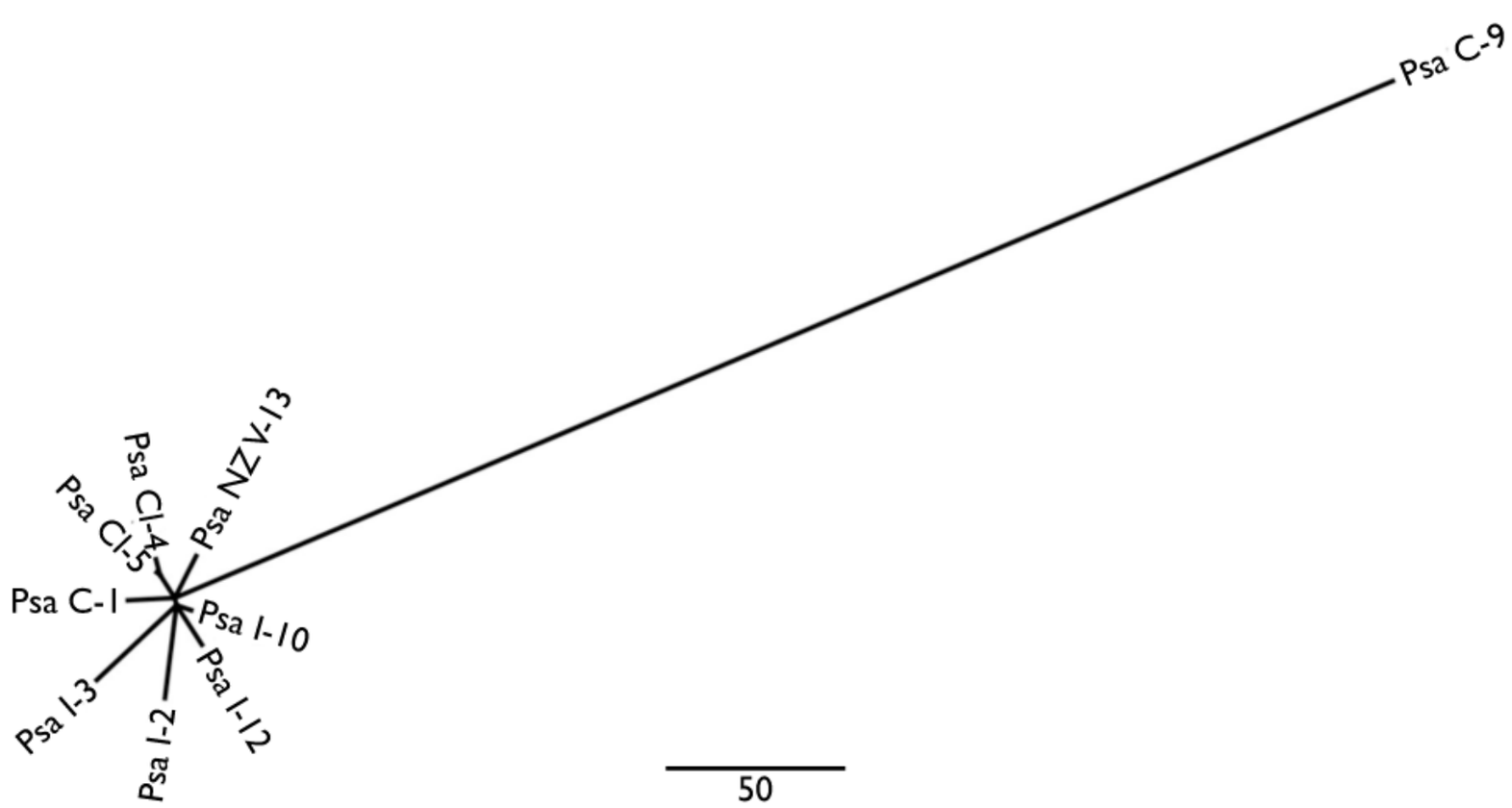
SNP analysis of the 2008 'global outbreak'

- Previous studies identified <10 SNPs...
- Read mapping to NZ V-13 generated ~1,000 SNPs
- Most SNPs are the product of recombination

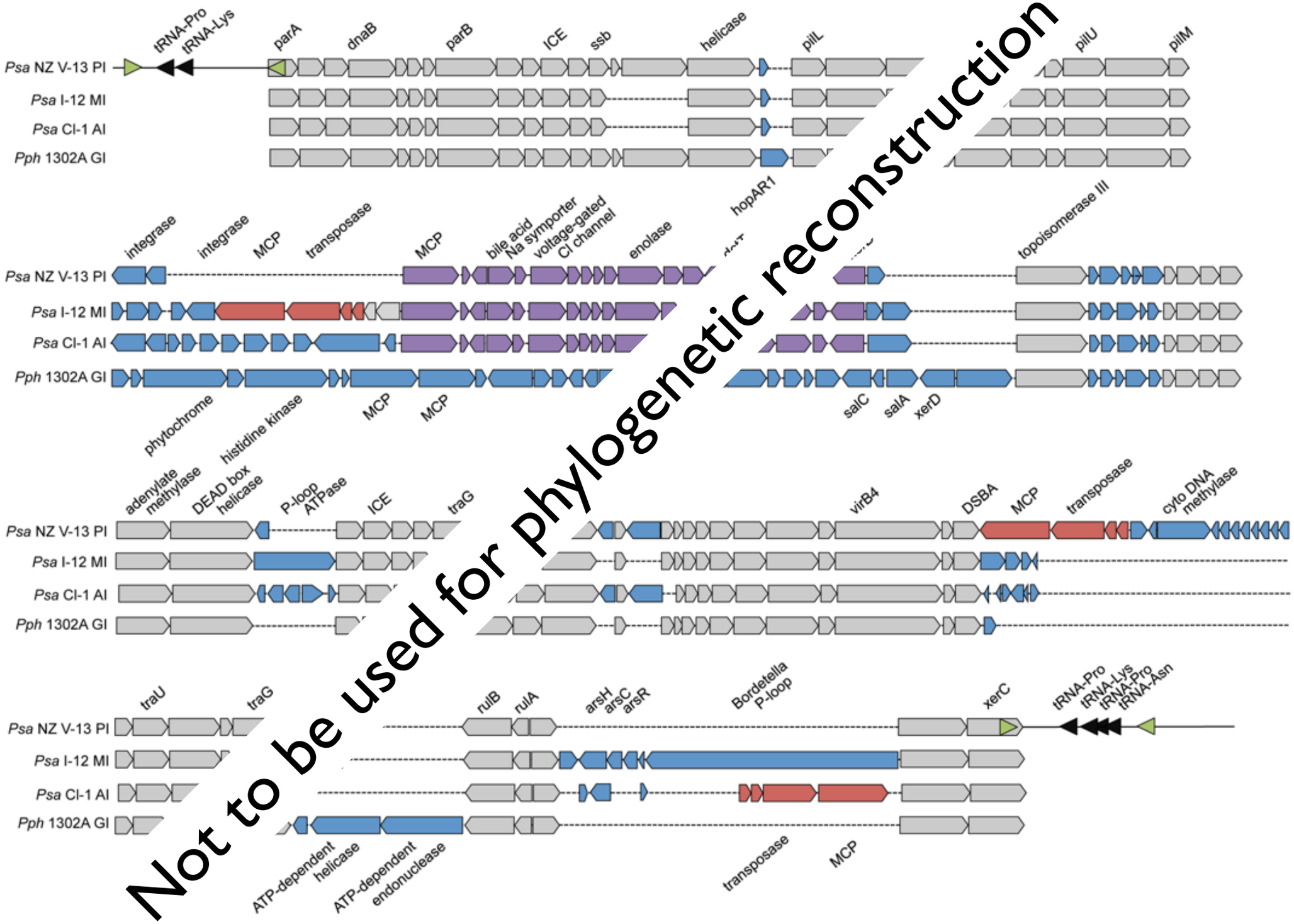
	Psa I-2	*Psa* I-3	*Psa* I-10	*Psa* I-12	*Psa* CI-4	*Psa* CI-5	*Psa* V-13	*Psa* C-1	*Psa* C-9[1]
Psa I-2	0	52	28	36	37	34	38	38	365
Psa I-3	71 (19)	0	32	40	41	38	42	42	369
Psa I-10	45 (17)	34 (2)	0	16	17	14	18	18	345
Psa I-12	60 (24)	49 (9)	23 (7)	0	25	22	26	26	353
Psa CI-4	115 (78)	104 (63)	78 (61)	93 (68)	0	5	23	23	350
Psa CI-5	112 (78)	101 (63)	75 (61)	90 (68)	5 (0)	0	20	20	347
Psa V-13	346 (308)	335 (293)	309 (291)	324 (298)	337 (314)	334 (314)	0	24	351
Psa C-1	345 (307)	334 (296)	308 (290)	323 (297)	336 (313)	333 (313)	25 (1)	0	351
Psa C-9	558 (193)	547 (178)	521 (176)	536 (183)	587 (237)	584 (237)	818 (467)	817 (466)	0

A star-like phylogeny (uninformative)

- Divergence within last 10 years
- Most polymorphism among Italian strains



Integrative conjugative elements (ICEs)



Conclusions

- Source population
  - Associated with wild *Actinidia* in south east Asia?
- Disease outbreaks a consequence of independent samplings
  - Opportunity? Anthropomorphic factors?
  - Implications for disease control and plant breeding
- 2008 outbreak
  - Polymorphism among Italian isolates
  - Introduction from south east Asia?
  - Opportunity due to gold kiwifruit plantings?
  - Dissemination from Italy
- Extraordinary evolutionary dynamics associated with ICEs
  - Cautionary notes with regard to phylogenetic inferences

Future work

- Evolutionary origins (source population)
- Evolutionary dynamics (spatial and temporal population (genomic) analyses)
- Evolution of virulence
- Functional studies (genetics)
- Interface with breeding programmes

Thank you

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