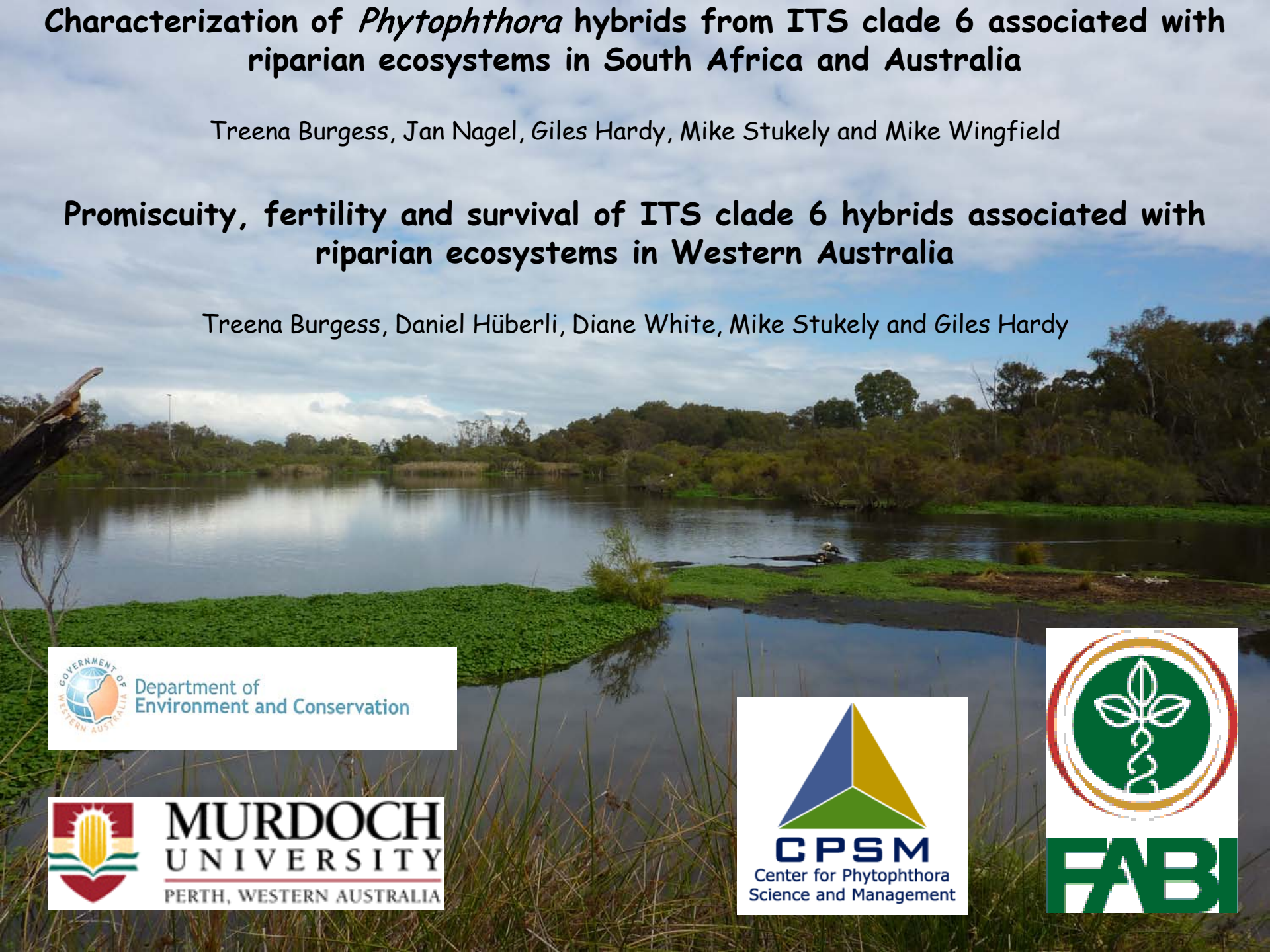
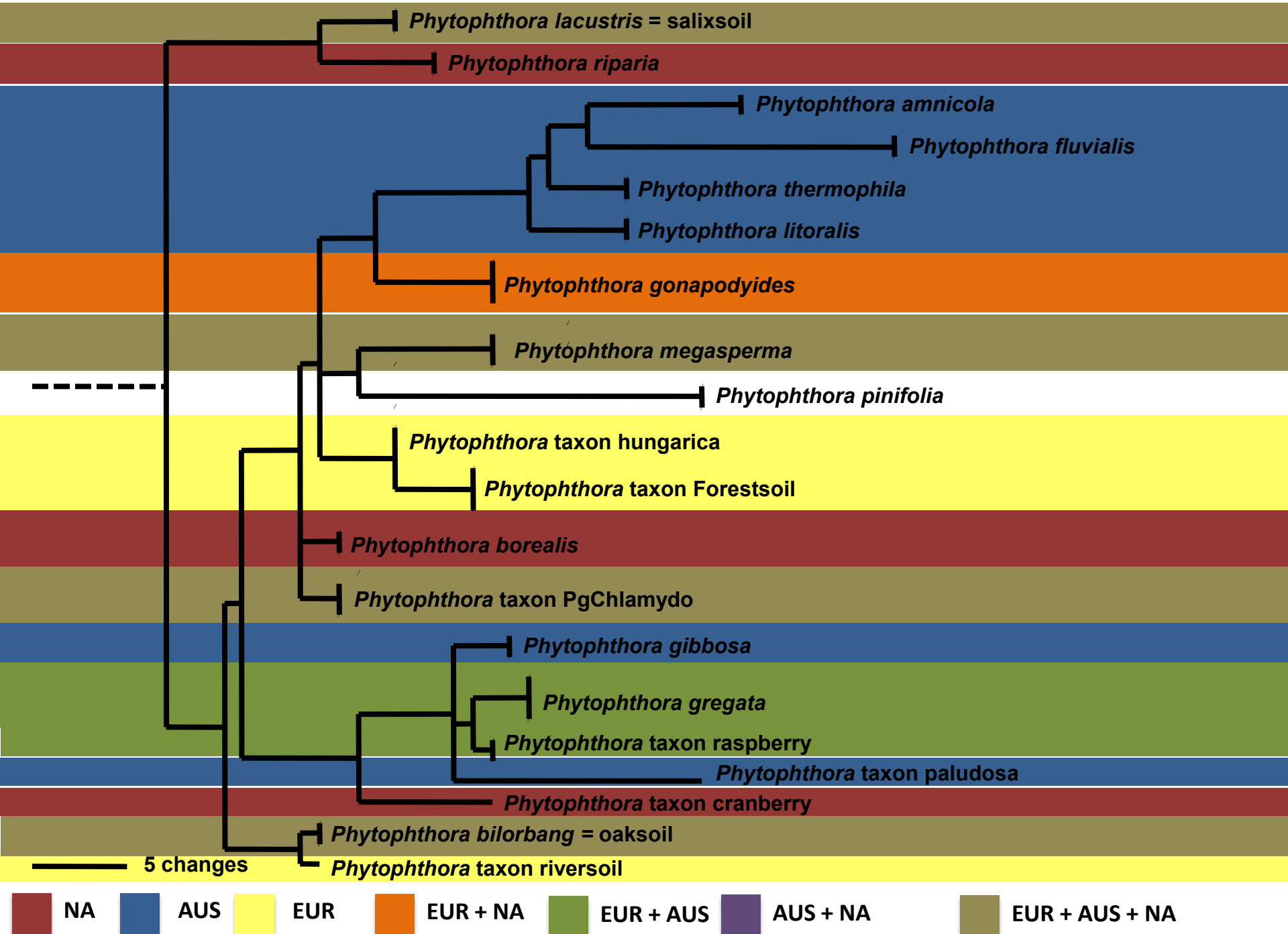
Characterization of *Phytophthora* hybrids from ITS clade 6 associated with riparian ecosystems in South Africa and Australia

Treena Burgess, Jan Nagel, Giles Hardy, Mike Stukely and Mike Wingfield

Promiscuity, fertility and survival of ITS clade 6 hybrids associated with riparian ecosystems in Western Australia

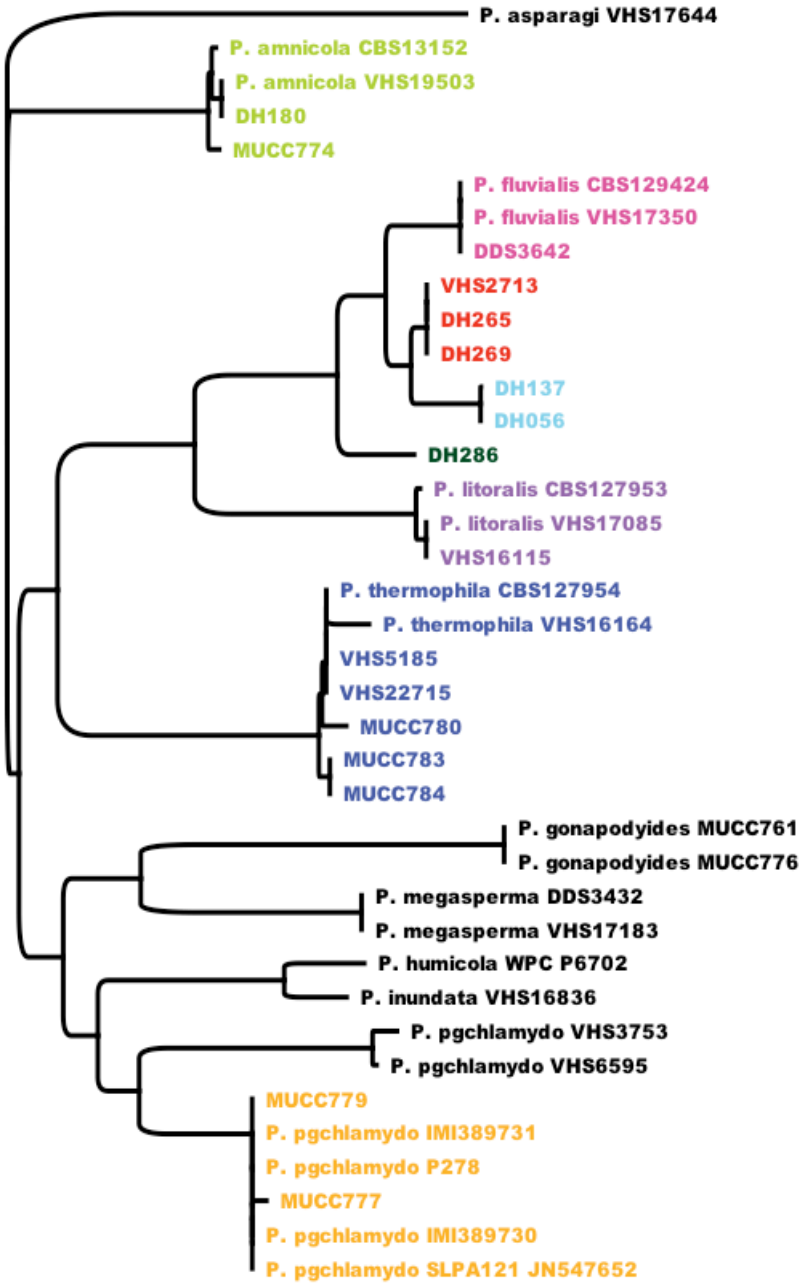
Treena Burgess, Daniel Hüberli, Diane White, Mike Stukely and Giles Hardy



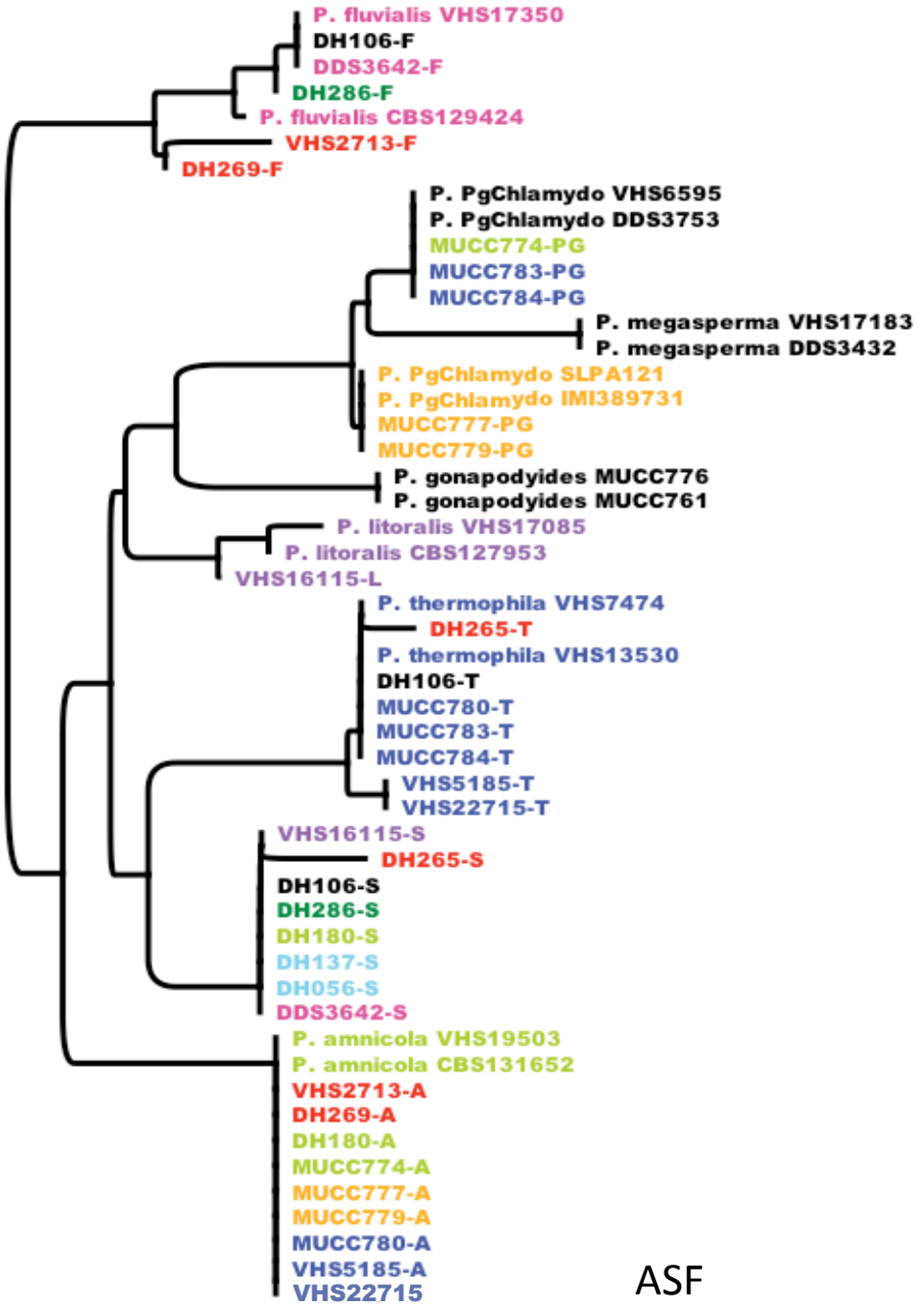

Phytophthora lacustris = salixsoil
Phytophthora riparia
Phytophthora amnicola
Phytophthora fluvialis
Phytophthora thermophila
Phytophthora litoralis
Phytophthora gonapodyides
Phytophthora megasperma
Phytophthora pinifolia
Phytophthora taxon hungarica
Phytophthora taxon Forestsoil
Phytophthora borealis
Phytophthora taxon PgChlamydo
Phytophthora gibbosa
Phytophthora gregata
Phytophthora taxon raspberry
Phytophthora taxon paludosa
Phytophthora taxon cranberry
Phytophthora bilorbang = oaksoil
Phytophthora taxon riversoil
5 changes
NA
AUS
EUR
EUR + NA
EUR + AUS
AUS + NA
EUR + AUS + NA

- in 2008 when conducting molecular characterisation of the VHS collection of the Department of Conservation we had several isolates that could not be identified using conventional sequencing of the ITS region because of numerous sites exhibiting additivity (double peaks) and severely truncated sequence data.
- at the time we thought they may be hybrids
- in 2009, Daniel Hüberli conducted his 'Fishing for Phytophthora' survey in 40 natural rivers and waterways in the south-west of Western Australia. Over half the ITS sequences failed
- at the same time Bill Dunstan conducted a survey along a proposed pipeline within natural forest in Victoria and Tim Rudman sampled highland rivers in Tasmania.
- from this we ended up with a large set of isolates that could not be identified by ITS sequence, were fast growing, had high temperature optima and maxima and were sterile.

- the next step was then to commence molecular characterisation.
- the ITS region was cloned and 10-50 amplicons sequenced for each isolate
- the *cox*I mitochondrial gene region was amplified and if any evidence of additivity it was cloned before sequencing
- the single copy genes used in the *P. alni* study of Ioos et al (2006) were all tested using a panel of known species from ITS clade 6, some primers were redesigned and finally we had reliable amplification using ASF-like (anti-silencing factor) and GPA1 (G-protein alpha subunit). Both regions were cloned and 6-12 amplicons sequenced


P. asparagi VHS17644
P. amnicola CBS13152
P. amnicola VHS19503
DH180
MUCC774
P. fluvialis CBS129424
P. fluvialis VHS17350
DDS3642
VHS2713
DH265
DH269
DH137
DH056
DH286
P. litoralis CBS127953
P. litoralis VHS17085
VHS16115
P. thermophila CBS127954
P. thermophila VHS16164
VHS5185
VHS22715
MUCC780
MUCC783
MUCC784
P. gonapodyides MUCC761
P. gonapodyides MUCC776
P. megasperma DDS3432
P. megasperma VHS17183
P. humicola WPC P6702
P. inundata VHS16836
P. pgchlamydo VHS3753
P. pgchlamydo VHS6595
MUCC779
P. pgchlamydo IMI389731
P. pgchlamydo P278
MUCC777
P. pgchlamydo IMI389730
P. pgchlamydo SLPA121 JN547652

cox


P. fluvialis VHS17350
DH106-F
DDS3642-F
DH286-F
P. fluvialis CBS129424
VHS2713-F
DH269-F
P. PgChlamydo VHS6595
P. PgChlamydo DDS3753
MUCC774-PG
MUCC783-PG
MUCC784-PG
P. megasperma VHS17183
P. megasperma DDS3432
P. PgChlamydo SLPA121
P. PgChlamydo IMI389731
MUCC777-PG
MUCC779-PG
P. gonapodyides MUCC776
P. gonapodyides MUCC761
P. litoralis VHS17085
P. litoralis CBS127953
VHS16115-L
P. thermophila VHS7474
DH265-T
P. thermophila VHS13530
DH106-T
MUCC780-T
MUCC783-T
MUCC784-T
VHS5185-T
VHS22715-T
VHS16115-S
DH265-S
DH106-S
DH286-S
DH180-S
DH137-S
DH056-S
DDS3642-S
P. amnicola VHS19503
P. amnicola CBS131652
VHS2713-A
DH269-A
DH180-A
MUCC774-A
MUCC777-A
MUCC779-A
MUCC780-A
VHS5185-A
VHS22715

ASF

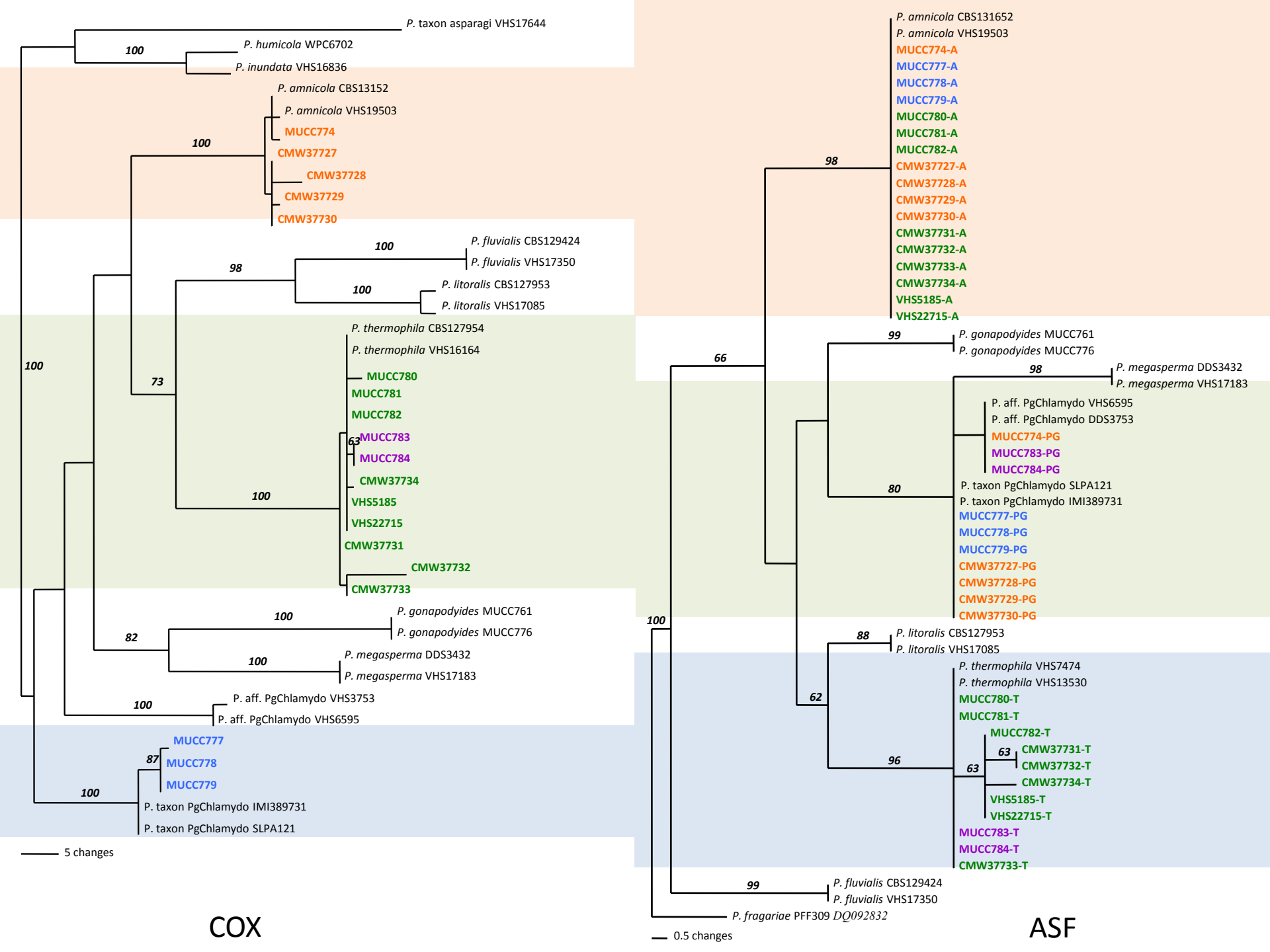
- during research collaboration in South Africa I was asked to assist a Jan Nagel who was working on a 'Fishing for Phytophthora' project
- he had many isolated exhibiting additivity in the ITS region and he had cloned and sequenced ITS amplicons and also *cox*I
- I realised that he had some of the same 'hybrid' types as I had found in Australia.
- this discovery actually simplified the story as I split the Australian hybrid isolates into two groups;
    1. those predominately from eastern Australia (and RSA)
    2. those exclusively from Western Australia

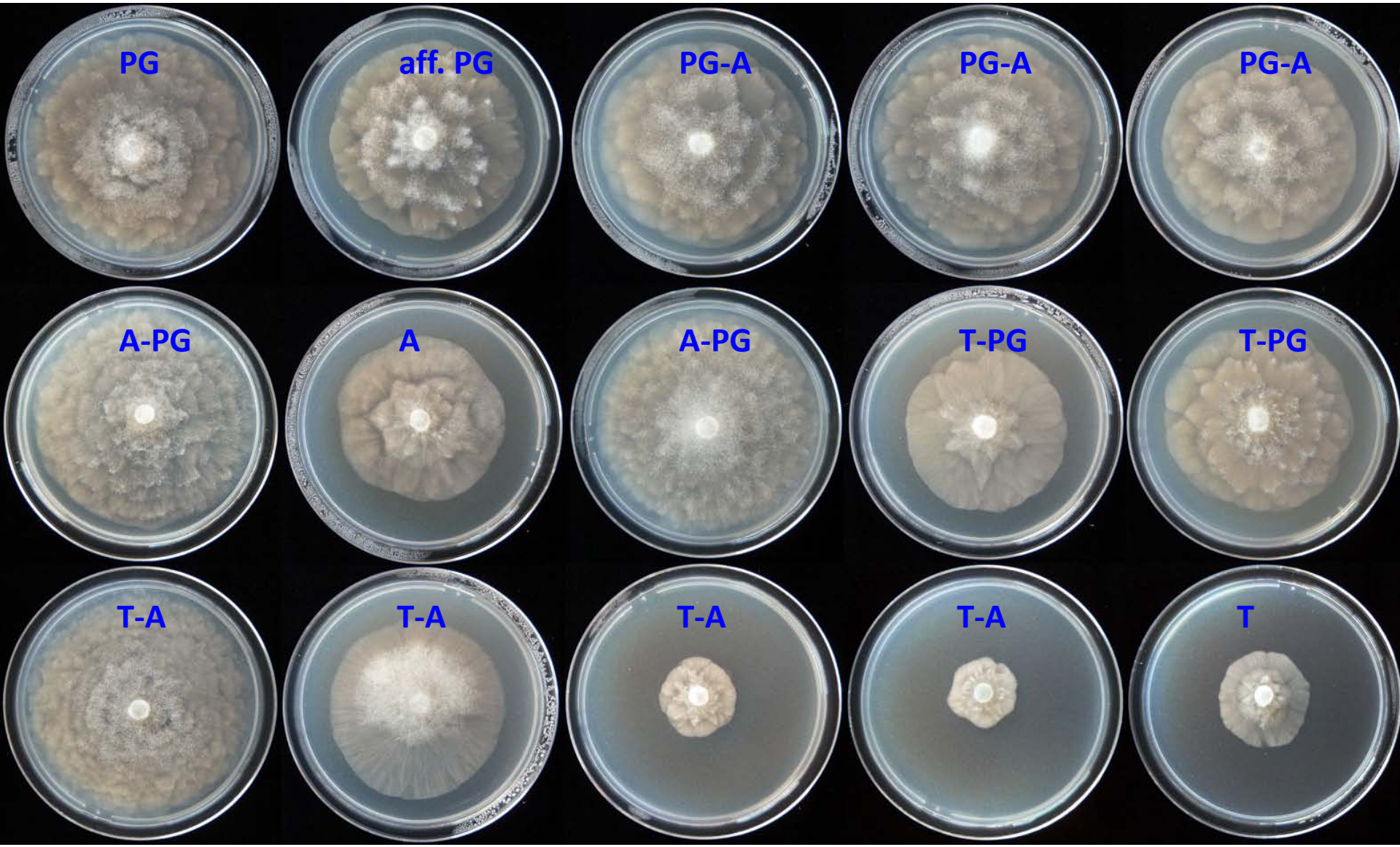
	41	44	59	71	110	148	162	171	180	184	457	476	477	518	519	555	699	746	748	753	788	815	
AMNICOLA	C	T	T	C	G	G	C	T	A	T	A	G	T	T	C	C	A	A	A	T	T	T	
A-PG		W	Y		R	R	M	N	W		R	S	K	Y	Y		R	R	R	Y		Y	17
PGCHLAMYDO	C	A	C	C	A	A	A	-	T	T	G	C	G	C	T	C	G	G	G	C	C	C	
PG-T	Y			Y	R	R	M		W	W	R			Y	Y	Y	R		R			Y	14
THERMOPHILA	T	A	C	T	G	G	C	-	A	A	A	C	G	T	C	T	A	G	A	C	C	T	
A-T	Y	W	Y	Y				N		W		S	K			Y		R		Y	Y		12

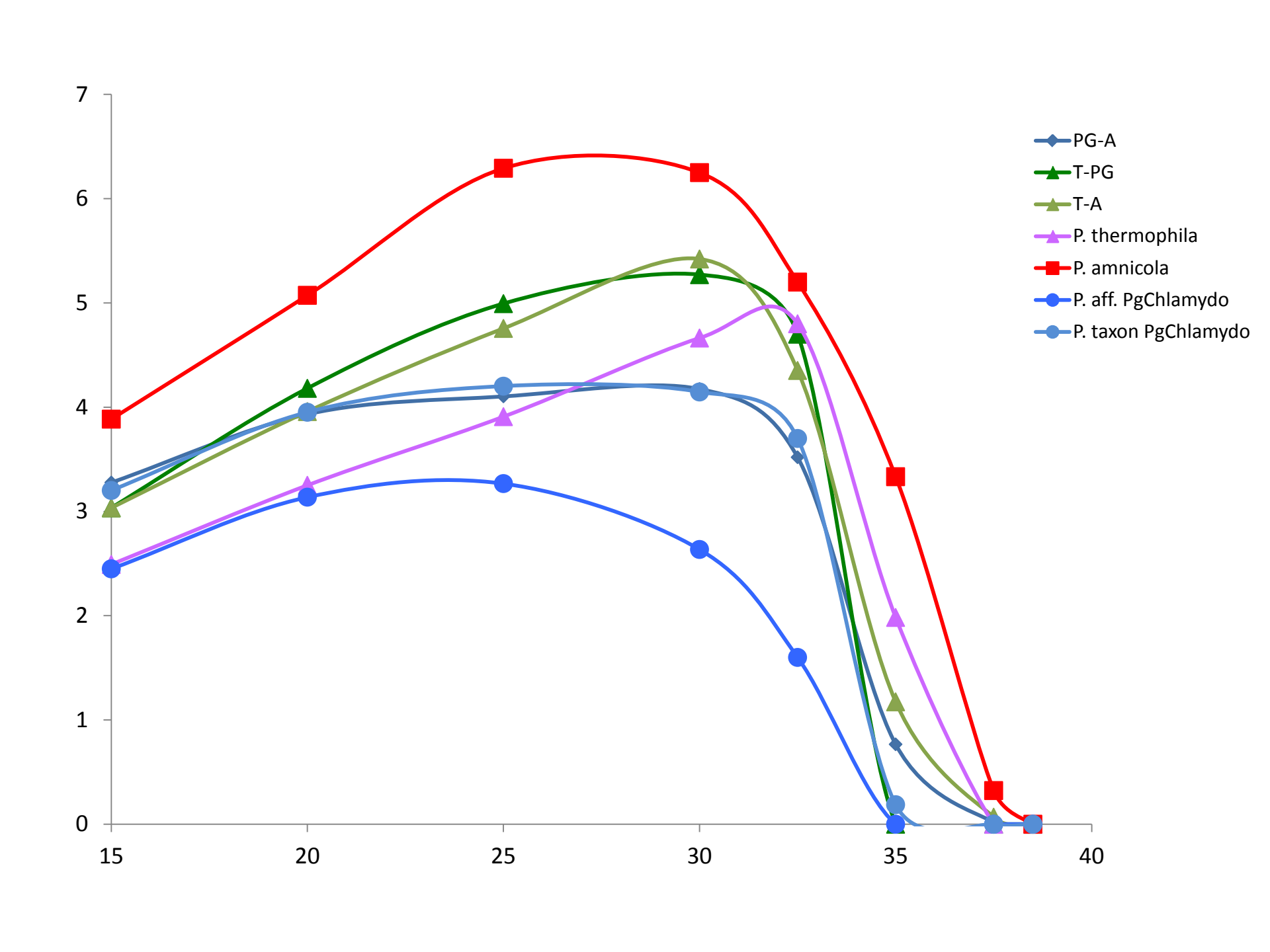
	n	41	44	59	71	110	148	162	171	180	184	457	476	477	518	519	555	699	746	748	753	788	815
A-PG			W	Y		R	R	M	N	W		R	S	K	Y	Y		R	R	R	Y		Y
CMW37727-1	2	C	A	C	C	A	A	A	-	T	T	G	C	G	C	T	C	G	G	G	C	C	C
CMW37727-2	2	C	T	T	C	G	G	C	T	A	T	G	C	G	C	T	C	A	A	A	T	C	T
CMW37727-3	1	C	A	C	C	A	A	A	-	T	T	G	C	G	C	T	C	A	A	A	T	C	T
CMW37727-4	5	C	T	T	C	G	G	C	T	A	T	A	G	T	T	C	C	A	A	A	T	C	T
CMW37728-1	1	C	A	C	C	A	A	A	-	T	T	G	C	G	C	T	C	G	G	G	C	C	C
CMW37728-2	9	C	T	T	C	G	G	C	T	A	T	A	G	T	T	C	C	A	A	A	T	G	T
CMW37729-1	4	C	T	T	C	G	G	C	T	A	T	A	G	T	T	C	C	A	A	A	T	G	T
CMW37729-2	2	C	T	T	C	G	G	A	-	T	T	G	G	T	T	C	C	A	A	A	T	C	T
CMW37729-3	2	C	A	C	C	A	A	A	-	T	T	A	G	T	T	C	C	A	A	A	T	T	T
CMW37730-1	2	C	A	C	C	A	A	A	-	T	T	G	C	G	C	T	C	G	G	G	C	C	C
CMW37730-2	1	C	A	C	C	A	G	C	T	A	T	A	G	T	T	C	C	A	A	A	T	T	T
CMW37730-3	1	C	T	T	C	G	G	C	T	A	T	G	C	G	C	T	C	G	G	G	C	C	C
CMW37730-4	6	C	T	T	C	G	G	C	T	A	T	A	G	T	T	C	C	A	A	A	T	T	T
MUCC777-1	2	C	A	C	C	A	A	A	-	T	T	G	C	G	C	T	C	G	G	G	C	C	C
MUCC777-2	1	C	A	C	C	A	A	A	-	T	T	G	C	G	C	T	C	A	A	A	T	C	T
MUCC777-3	1	C	T	T	C	G	G	C	T	A	T	A	G	T	T	C	C	A	G	G	C	C	C
MUCC777-4	6	C	T	T	C	G	G	C	T	A	T	A	G	T	T	C	C	A	A	A	T	C	T
MUCC778-1	6	C	A	C	C	A	A	A	-	T	T	G	C	G	C	T	C	G	G	G	C	C	C
MUCC778-2	2	C	A	C	C	A	A	A	-	T	T	G	C	G	C	T	C	A	A	A	T	C	T
MUCC778-3	2	C	T	T	C	G	G	C	T	A	T	A	G	T	T	C	C	A	A	A	T	C	T
MUCC779-1	1	C	A	C	C	A	A	A	-	T	T	G	C	G	C	T	C	G	G	G	C	C	G
MUCC779-2	1	C	A	C	C	A	A	C	T	A	T	G	C	G	C	T	C	G	G	G	C	C	T
MUCC779-3	2	C	T	T	C	G	G	C	T	A	T	A	G	T	T	C	C	G	G	G	C	C	C
MUCC779-4	6	C	T	T	C	G	G	C	T	A	T	A	G	T	T	C	C	A	A	A	T	C	T
MUCC774-1	1	C	A	C	C	A	A	A	-	T	T	G	C	G	C	T	C	G	G	G	C	C	C
MUCC774-2	1	C	A	C	C	A	A	A	-	T	T	G	C	G	T	C	C	A	A	A	T	T	T
MUCC774-3	1	C	T	T	C	G	G	C	T	A	T	G	C	G	C	T	C	G	G	G	C	C	C
MUCC774-4	1	C	A	C	C	A	A	A	-	T	T	A	G	T	T	C	C	G	G	G	C	C	C
MUCC774-5	1	C	T	T	C	G	G	C	T	A	T	A	G	T	T	C	C	G	G	G	C	C	C
MUCC774-6	1	C	A	C	C	A	A	A	-	T	T	G	C	G	T	C	C	A	A	A	T	T	T
MUCC774-7	1	C	T	T	C	G	G	C	T	A	T	G	C	G	C	T	C	G	G	G	C	C	C
MUCC774-8	1	C	T	T	C	G	G	C	-	T	T	G	C	G	C	T	C	G	G	G	C	C	C
MUCC774-9	2	C	T	T	C	G	G	C	T	A	T	A	G	T	T	C	C	A	A	A	T	C	T

	n	41	44	59	71	110	148	162	171	180	184	457	476	477	518	519	555	699	746	748	753	788	815
PG-T		Y			Y	R	R	M		W	W	R			Y	Y	Y	R		R			Y
MUCC783-1	2	C	A	C	C	A	G	C	-	A	A	A	C	G	T	C	T	A	G	A	C	C	C
MUCC783-2	8	C	A	C	C	A	A	A	-	T	T	G	C	G	C	T	C	G	G	G	C	C	C
MUCC784-1	2	T	A	C	T	G	G	C	-	A	A	A	C	G	T	C	T	A	G	A	C	C	T
MUCC784-2	8	C	A	C	C	A	A	A	-	T	T	G	C	G	C	T	C	G	G	G	C	C	C

	n	41	44	59	71	110	148	162	171	180	184	457	476	477	518	519	555	699	746	748	753	788	815
A-T		Y	W	Y	Y				N		W		S	K			Y		R		Y	Y	
CMW37731-1	8	C	T	T	C	G	G	C	T	A	T	A	G	T	T	C	C	A	A	A	T	C	T
CMW37731-2	2	T	A	C	T	G	G	C	-	A	A	A	C	G	T	C	T	A	G	A	C	C	T
CMW37732-1	9	C	T	T	C	G	G	C	T	A	T	A	G	T	T	C	C	A	A	A	T	C	T
CMW37732-2	1	C	T	C	C	G	G	T	T	A	T	A	G	T	T	C	C	A	G	A	C	C	T
MUCC780-1	9	C	T	T	C	G	G	C	T	A	T	A	G	T	T	C	C	A	A	A	T	G	T
MUCC780-2	1	T	A	C	T	G	G	C	-	A	A	A	G	T	T	C	C	A	A	A	T	G	T
CMW37733-1	5	C	T	T	C	G	G	C	T	A	T	A	G	T	T	C	C	A	A	A	T	C	T
CMW37733-2	1	T	A	C	T	G	G	C	-	A	A	A	G	T	T	C	C	A	A	A	T	C	T
CMW37733-3	1	C	T	T	C	G	G	C	T	A	T	A	G	T	T	C	C	A	G	A	C	C	T
CMW37733-4	1	T	A	C	T	G	G	C	-	A	A	A	G	T	T	C	C	A	A	A	T	C	T
CMW37733-5	1	T	A	C	T	G	G	C	-	A	A	A	C	G	T	C	T	A	A	A	T	C	T
CMW37733-6	1	C	T	T	C	G	G	C	T	A	T	A	C	G	T	C	C	A	A	A	T	C	T
CMW37734-1	4	C	T	T	C	G	G	C	T	A	T	A	G	T	T	C	C	A	A	A	T	G	T
CMW37734-2	2	T	A	C	T	G	G	C	T	A	T	A	G	T	T	C	C	A	A	A	T	G	T
CMW37734-3	1	T	A	C	C	G	G	C	T	A	T	A	G	T	T	C	C	A	A	A	T	T	T
CMW37734-4	3	T	A	C	T	G	G	C	-	A	A	A	C	G	T	C	T	A	G	A	C	C	T
MUCC781-1	5	C	T	T	C	G	G	C	T	A	T	A	G	T	T	C	C	A	A	A	T	G	T
MUCC781-2	2	C	T	T	C	G	G	C	T	A	T	A	G	T	T	C	C	A	G	A	T	C	T
MUCC781-3	1	C	T	T	C	G	G	C	T	A	T	A	C	G	T	C	T	A	G	A	C	C	T
MUCC781-4	2	T	A	C	T	G	G	C	-	A	A	A	C	G	T	C	T	A	G	A	C	C	T
MUCC782-1	3	C	T	T	C	G	G	C	T	A	T	A	G	T	T	C	C	A	A	A	T	T	T
MUCC782-2	1	T	A	T	C	G	G	C	T	A	T	A	G	T	T	C	C	A	A	A	T	C	T
MUCC782-3	2	T	A	C	T	G	G	C	-	A	A	A	C	G	T	C	C	A	A	A	T	T	T
MUCC782-4	1	C	T	C	T	G	G	C	T	A	T	A	G	T	T	C	C	A	A	A	T	T	T
MUCC782-5	1	T	A	C	T	G	G	C	-	A	A	A	G	T	T	C	C	A	A	A	T	C	T
MUCC782-6	2	T	A	C	T	G	G	C	-	A	A	A	C	G	T	C	T	A	G	A	C	C	T
VHS22715-1	7	C	T	T	C	G	G	C	T	A	T	A	G	T	T	C	C	A	A	A	T	T	T
VHS22715-2	1	C	T	T	C	G	G	C	T	A	T	A	C	G	T	C	T	A	A	A	T	G	T
VHS22715-3	1	C	T	T	C	G	G	C	T	A	T	A	C	G	T	C	T	A	G	A	C	C	T
VHS22715-4	1	C	T	T	C	G	G	C	-	A	A	A	C	G	T	C	T	A	G	A	C	C	T
VHS5185-1	6	C	T	T	C	G	G	C	T	A	T	A	G	T	T	C	C	A	A	A	T	T	T
VHS5185-2	1	C	T	T	C	G	G	C	T	A	T	A	C	G	T	C	T	A	G	A	C	C	T
VHS5185-3	1	C	T	T	C	G	G	C	T	A	T	A	C	G	T	C	C	A	A	A	T	T	T
VHS5185-4	1	C	T	T	C	G	G	C	T	A	T	A	C	G	T	C	T	A	A	A	T	T	T
VHS5185-5	1	C	T	C	T	G	G	C	-	A	A	A	C	G	T	C	T	A	A	A	T	T	T


P. taxon asparagi VHS17644
P. humicola WPC6702
P. inundata VHS16836
P. amnicola CBS13152
P. amnicola VHS19503
MUCC774
CMW37727
CMW37728
CMW37729
CMW37730
P. fluvialis CBS129424
P. fluvialis VHS17350
P. litoralis CBS127953
P. litoralis VHS17085
P. thermophila CBS127954
P. thermophila VHS16164
MUCC780
MUCC781
MUCC782
MUCC783
MUCC784
CMW37734
VHS5185
VHS22715
CMW37731
CMW37732
CMW37733
P. gonapodyides MUCC761
P. gonapodyides MUCC776
P. megasperma DDS3432
P. megasperma VHS17183
P. aff. PgChlamydo VHS3753
P. aff. PgChlamydo VHS6595
MUCC777
MUCC778
MUCC779
P. taxon PgChlamydo IMI389731
P. taxon PgChlamydo SLPA121
5 changes
COX
P. amnicola CBS131652
P. amnicola VHS19503
MUCC774-A
MUCC777-A
MUCC778-A
MUCC779-A
MUCC780-A
MUCC781-A
MUCC782-A
CMW37727-A
CMW37728-A
CMW37729-A
CMW37730-A
CMW37731-A
CMW37732-A
CMW37733-A
CMW37734-A
VHS5185-A
VHS22715-A
P. gonapodyides MUCC761
P. gonapodyides MUCC776
P. megasperma DDS3432
P. megasperma VHS17183
P. aff. PgChlamydo VHS6595
P. aff. PgChlamydo DDS3753
MUCC774-PG
MUCC783-PG
MUCC784-PG
P. taxon PgChlamydo SLPA121
P. taxon PgChlamydo IMI389731
MUCC777-PG
MUCC778-PG
MUCC779-PG
CMW37727-PG
CMW37728-PG
CMW37729-PG
CMW37730-PG
P. litoralis CBS127953
P. litoralis VHS17085
P. thermophila VHS7474
P. thermophila VHS13530
MUCC780-T
MUCC781-T
MUCC782-T
CMW37731-T
CMW37732-T
CMW37734-T
VHS5185-T
VHS22715-T
MUCC783-T
MUCC784-T
CMW37733-T
P. fluvialis CBS129424
P. fluvialis VHS17350
P. fragariae PFF309 DQ092832
0.5 changes
ASF


PG
aff. PG
PG-A
PG-A
PG-A
A-PG
A
A-PG
T-PG
T-PG
T-A
T-A
T-A
T-A
T


7
6
5
4
3
2
1
0
15
20
25
30
35
40
PG-A
T-PG
T-A
P. thermophila
P. amnicola
P. aff. PgChlamydo
P. taxon PgChlamydo

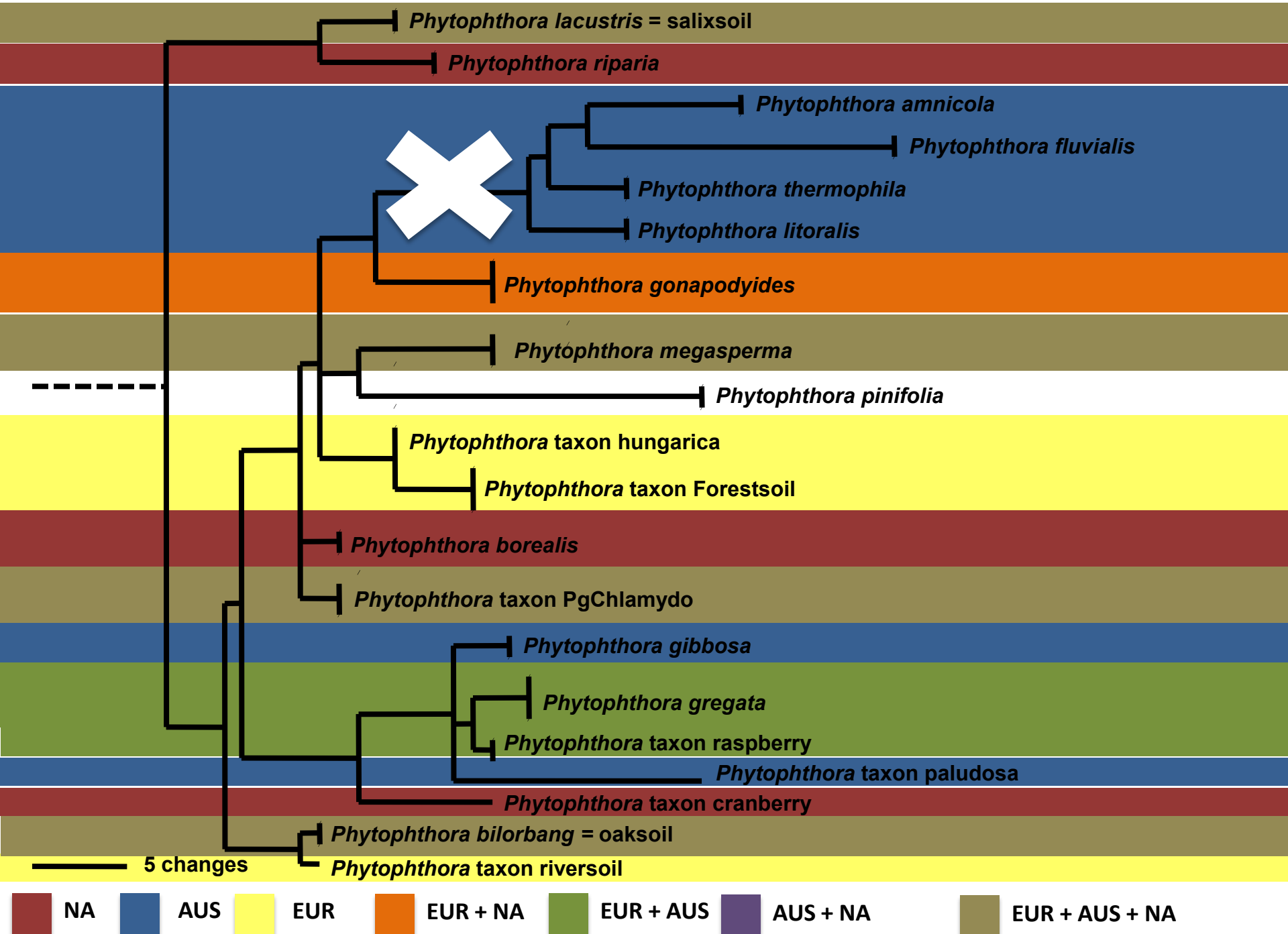
	P. thermophila	P. amnicola	P. PgChlamydo	PG-A	A-PG	T-A	T-PG
Sporangia shape	ovoid	ovoid	ovoid	ovoid	ovoid	ovoid	limoniform
Sporangia size	45 x25	62 x35	57 x 35	56 x 34	39 x 27	48 x 30	48 x 31
Sexual system	Sterile or silent homothallic	sterile ?	sterile ?	sterile	sterile	sterile	sterile
swellings	Globose or elongated, partly catenulate	Ellipsoid to irregular catenulate hyphal swellings in clusters	globose	globose	No swellings	No swellings	No swellings
chlamydo-spores	Globose, radiating hyphae		Globose, radiating hyphae	globose			
Temp max	35	37.5	35	>35<37.5	>35<37.5	>35<37.5	35

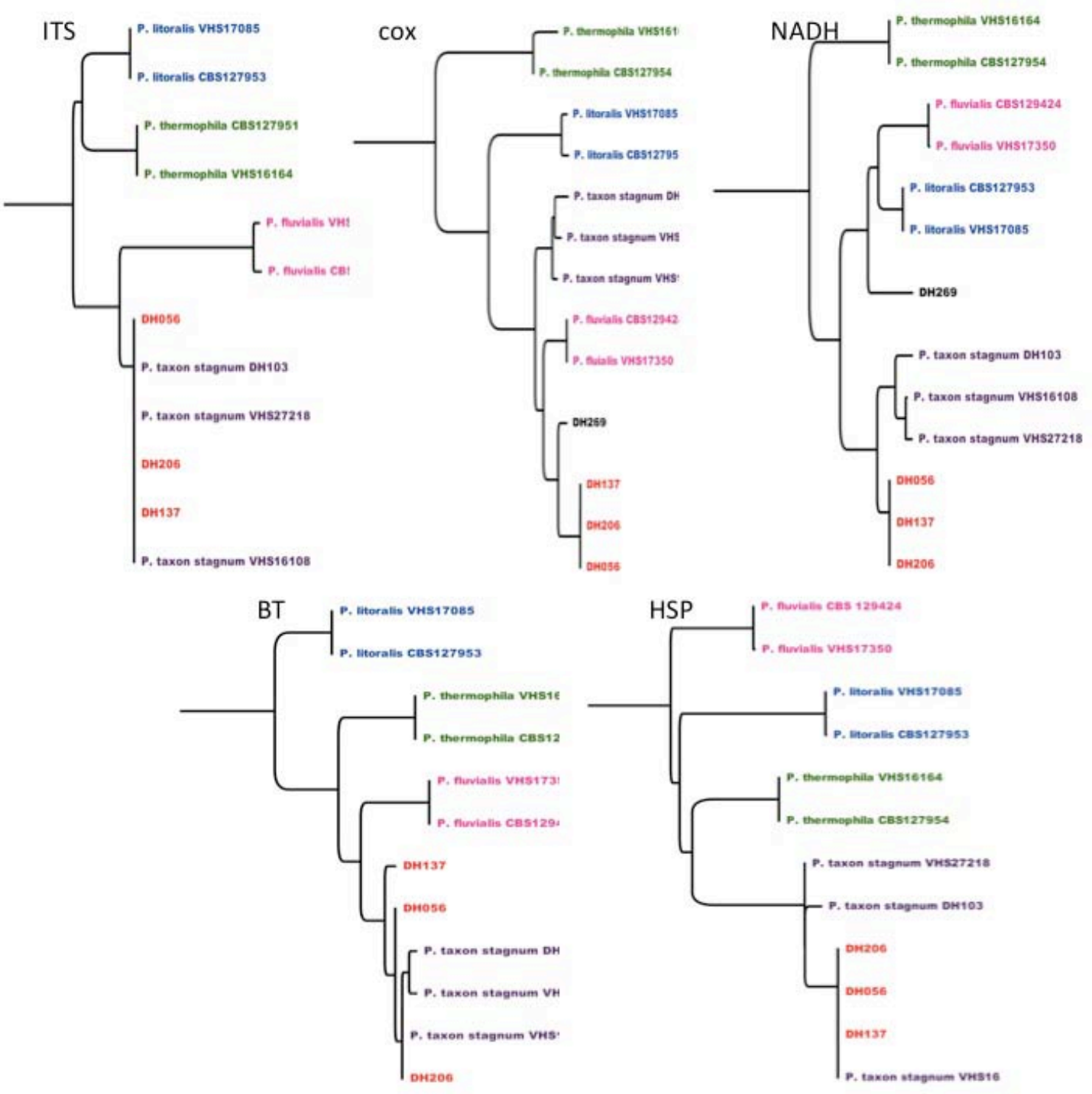
	WA	EA	RSA
P. thermophila	+++	+++	
P. amnicola	+++	?	
P. PgChlamydo		+++	
P. aff. PgChlamydo	+++	?	+++
PG-A		+++ (VIC)	+++
A-aff. PG		+++ (TAS)	
T-A	+++	+++ (TAS)	+++
T-aff. PG		+++ (TAS)	

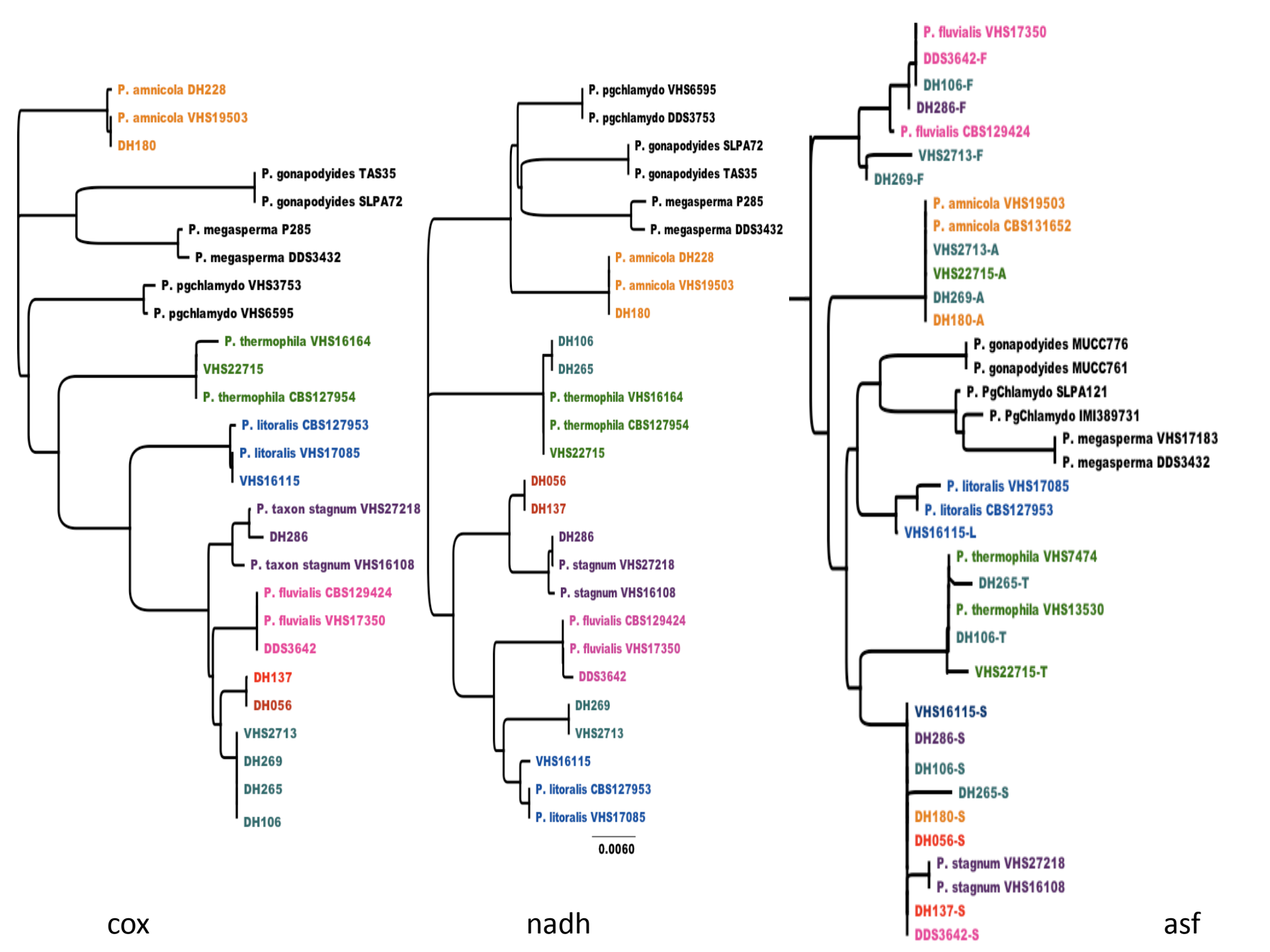
parental species and all hybrids in Australia

- In general, hybrid formation appears to be through sexual cross between two parental species. How?
- PgChlamydo and *P. amnicola* both appear to be sterile when mated with tester strains, but one isolate *P. thermophila* formed oospores after being left in soil extract. Are we unable to induce the right conditions in laboratory?
- all parental species and hybrids common in waterways.
    1. Is there a hybrid swarm perhaps through somatic hybridisation between sterile hybrids?
    2. can they back cross with parental species?
    3. or are hybrids constantly formed by new hybridisation events between parental species, but die out if conditions are unfavourable for their growth and survival
- how did they get to South Africa? are they found elsewhere in the world?

- these hybrids between amnicola, thermophila and PgChlamydo are widespread in all waterways examined to date in Australia and South Africa (except Western Australia)
- I then excluded these hybrids from the matrix and examined only 'hybrid' isolates from Western Australia (approx 50 isolates-many more available)
- they have been isolated predominantly from waterways BUT they have also been isolated in the rhizosphere soil of dying plants after inundation.
- for this presentation I have selected representative isolates of the different hybrid types


Phytophthora lacustris = salixsoil
Phytophthora riparia
Phytophthora amnicola
Phytophthora fluvialis
Phytophthora thermophila
Phytophthora litoralis
Phytophthora gonapodyides
Phytophthora megasperma
Phytophthora pinifolia
Phytophthora taxon hungarica
Phytophthora taxon Forestsoil
Phytophthora borealis
Phytophthora taxon PgChlamydo
Phytophthora gibbosa
Phytophthora gregata
Phytophthora taxon raspberry
Phytophthora taxon paludosa
Phytophthora taxon cranberry
Phytophthora bilorbang = oaksoil
Phytophthora taxon riversoil
5 changes
NA
AUS
EUR
EUR + NA
EUR + AUS
AUS + NA
EUR + AUS + NA


ITS
P. litoralis VHS17085
P. litoralis CBS127953
P. thermophila CBS127951
P. thermophila VHS16164
P. fluvialis VHS
P. fluvialis CBS
DH056
P. taxon stagnum DH103
P. taxon stagnum VHS27218
DH206
DH137
P. taxon stagnum VHS16108
cox
P. thermophila VHS161
P. thermophila CBS127954
P. litoralis VHS17085
P. litoralis CBS12795
P. taxon stagnum DH
P. taxon stagnum VHS
P. taxon stagnum VHS
P. fluvialis CBS12942
P. fluialis VHS17350
DH269
DH137
DH206
DH056
NADH
P. thermophila VHS16164
P. thermophila CBS127954
P. fluvialis CBS129424
P. fluvialis VHS17350
P. litoralis CBS127953
P. litoralis VHS17085
DH269
P. taxon stagnum DH103
P. taxon stagnum VHS16108
P. taxon stagnum VHS27218
DH056
DH137
DH206
BT
P. litoralis VHS17085
P. litoralis CBS127953
P. thermophila VHS16
P. thermophila CBS12
P. fluvialis VHS173
P. fluvialis CBS129
DH137
DH056
P. taxon stagnum DH
P. taxon stagnum VH
P. taxon stagnum VHS
DH206
HSP
P. fluvialis CBS 129424
P. fluvialis VHS17350
P. litoralis VHS17085
P. litoralis CBS127953
P. thermophila VHS16164
P. thermophila CBS127954
P. taxon stagnum VHS27218
P. taxon stagnum DH103
DH206
DH056
DH137
P. taxon stagnum VHS16


P. amnicola DH228
P. amnicola VHS19503
DH180
P. gonapodyides TAS35
P. gonapodyides SLPA72
P. megasperma P285
P. megasperma DDS3432
P. pgchlamydo VHS3753
P. pgchlamydo VHS6595
P. thermophila VHS16164
VHS22715
P. thermophila CBS127954
P. litoralis CBS127953
P. litoralis VHS17085
VHS16115
P. taxon stagnum VHS27218
DH286
P. taxon stagnum VHS16108
P. fluvialis CBS129424
P. fluvialis VHS17350
DDS3642
DH137
DH056
VHS2713
DH269
DH265
DH106
P. pgchlamydo VHS6595
P. pgchlamydo DDS3753
P. gonapodyides SLPA72
P. gonapodyides TAS35
P. megasperma P285
P. megasperma DDS3432
P. amnicola DH228
P. amnicola VHS19503
DH180
DH106
DH265
P. thermophila VHS16164
P. thermophila CBS127954
VHS22715
DH056
DH137
DH286
P. stagnum VHS27218
P. stagnum VHS16108
P. fluvialis CBS129424
P. fluvialis VHS17350
DDS3642
DH269
VHS2713
VHS16115
P. litoralis CBS127953
P. litoralis VHS17085
0.0060
P. fluvialis VHS17350
DDS3642-F
DH106-F
DH286-F
P. fluvialis CBS129424
VHS2713-F
DH269-F
P. amnicola VHS19503
P. amnicola CBS131652
VHS2713-A
VHS22715-A
DH269-A
DH180-A
P. gonapodyides MUCC776
P. gonapodyides MUCC761
P. PgChlamydo SLPA121
P. PgChlamydo IMI389731
P. megasperma VHS17183
P. megasperma DDS3432
P. litoralis VHS17085
P. litoralis CBS127953
VHS16115-L
P. thermophila VHS7474
DH265-T
P. thermophila VHS13530
DH106-T
VHS22715-T
VHS16115-S
DH286-S
DH106-S
DH265-S
DH180-S
DH056-S
P. stagnum VHS27218
P. stagnum VHS16108
DH137-S
DDS3642-S

cox nadh asf

	ITS CLONES	COX ID	NADH	ASF
VHS2713	A-S	2.5	2.5	A-F
269	A-S	2.5	2.5	A-F
VHS22715	T-A	P. thermophila	T	T-A
265	T-S	2.5	T	T-S
106	T-S	2.5	T	F-S-T
180	A-S	P. amnicola	A	A-S
286	S-F	P. taxon stagnum	S	S-F
DDS3642	F-S	P. fluvialis	F	F-S
VHS16115	S-L	P. litoralis	L	S-L
56	S	2.8	2.8	S
137	S	2.8	2.8	S

images T. Jung

- In WA we have evidence for hybrids forming with *P. amnicola, P. thermophila, P. litoralis, P. fluvialis* and P. taxon stagnum as parental species
- cox1 and NADH data suggest there may be two additional species not yet identified
- hybrids formed are; A-S, T-A, L-S, S-F, F-S
- based on ASF there is also S-A and S-F, but cox types unknown
- based on ASF alleles corresponding to F-S-T, may be some sort of backcrossing (sterile?) or somatic hybridisation
- some isolates have ITS and ASF like P. taxon stagnum but unknown cox and NADH types
- possibilities to explain observations include non-inheritance of alleles from one parent or mitotically unstable progeny (hybrids)
- next step will be flow cytometry to assess genome size, microsatellites would also be useful but difficult to design over several genera

P. gonapodyides and PgChlamydo not found in Western Australia