

Identification and characterization of *Phytophthora* hybrids using Genotyping-By-Sequencing

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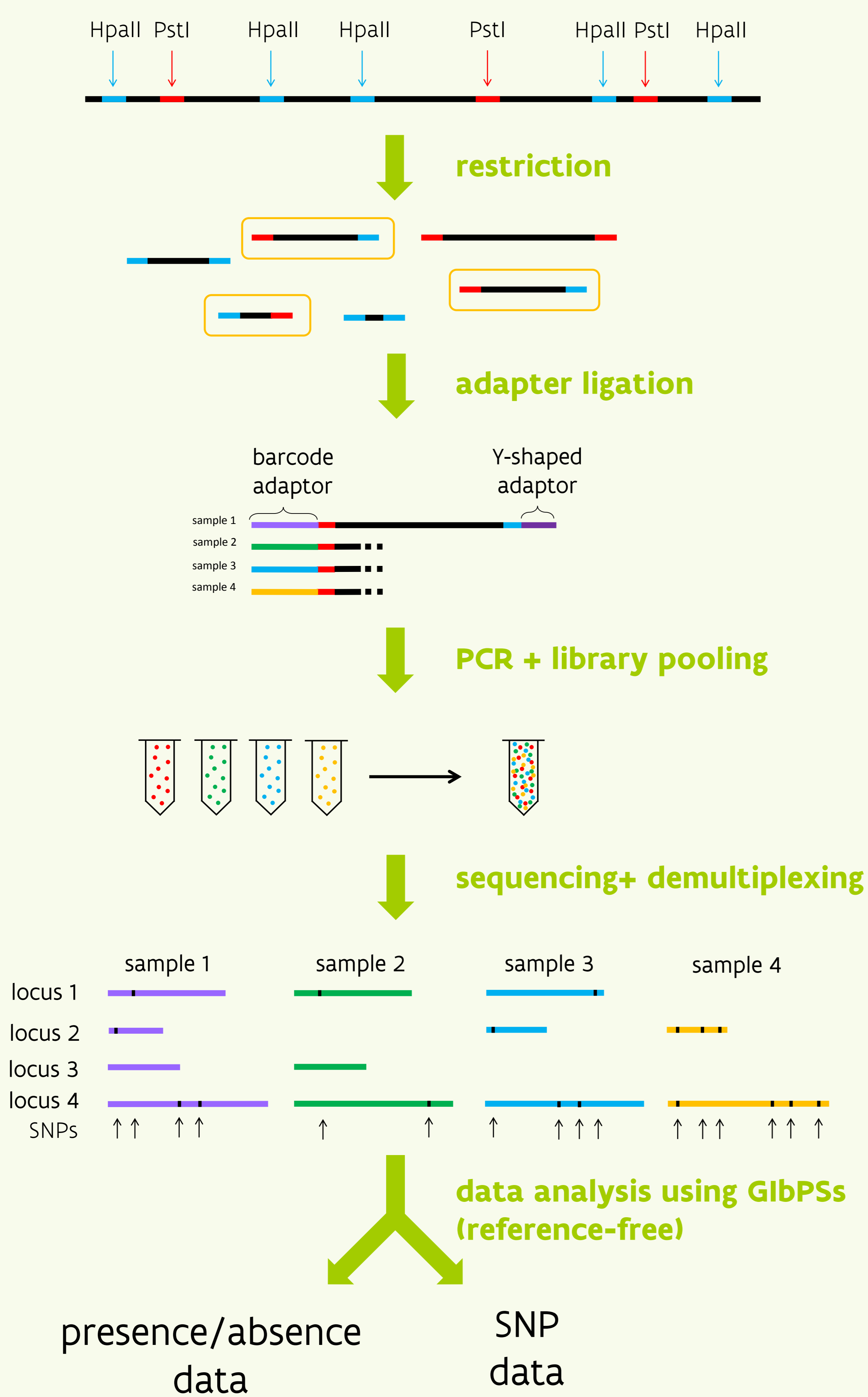
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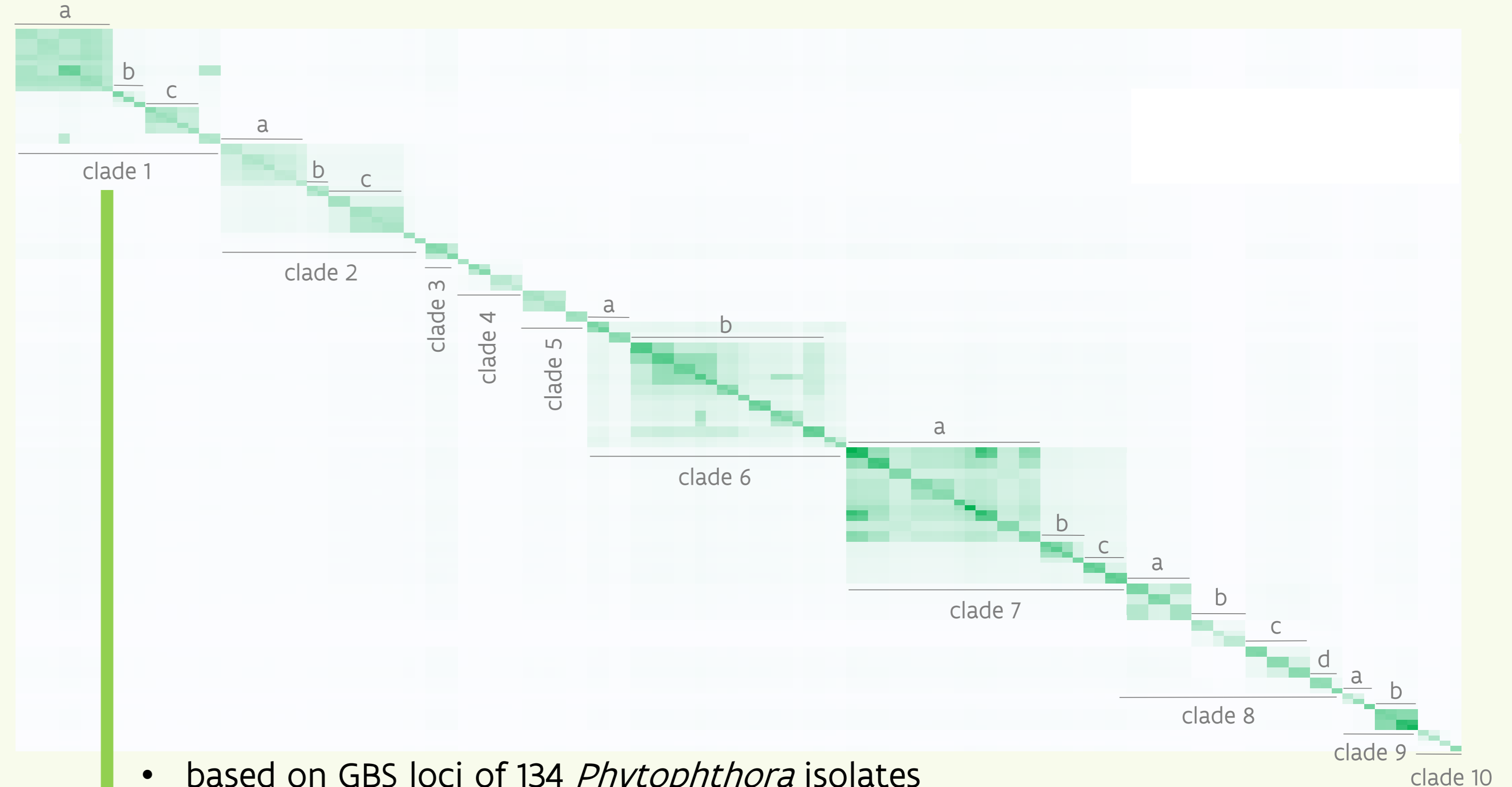
Introduction

Phytophthora is a genus that belongs to the Oomycetes and that contains many important plant pathogenic species. These are classified into 10 phylogenetic clades. In some clades, hybrids have been identified that can have an expanded host range and can be more aggressive compared to the parental species. A reliable method to distinguish between hybrids and their parental species is therefore very important. We have applied Genotyping-By-Sequencing as a tool for the identification and characterization of *Phytophthora* species and their hybrids.

Genotyping-by-sequencing (GBS)



Heatmap based on number of common GBS loci



- based on GBS loci of 134 *Phytophthora* isolates
- isolates from different clades share ~2-5% of loci (white)
- isolates from same clade but different subclade share ~5-10% of loci (pale green)
- isolates from same subclade share ~25-75% of loci (dark green)
- large number of loci (very dark green) are indicative of (allopolyploid) hybridization

Number of common GBS loci in clade 1 (1000x)

	cactorum 1	cactorum 2	hedraiaandra 1	hedraiaandra 2	cactorum x hedraiaandra 1	cactorum x hedraiaandra 2	idaei	cactorum x nicotiana 1	cactorum x nicotiana 2	iranica	clandestina	tentaculata	mirabilis	ipomoeae	andina (infestans hybrid)	infestans 1	infestans 2	nicotiana 1	nicotiana 2
cactorum 1	17,6	17,1	13,3	13,1	16,0	16,6	12,7	16,6	16,8	1,5	1,5	1,7	1,2	1,2	1,2	1,1	1,1	1,7	1,7
cactorum 2	17,1	17,5	13,3	13,0	16,2	16,8	12,7	16,9	16,8	1,5	1,5	1,7	1,2	1,2	1,2	1,1	1,1	1,7	1,7
hedraiaandra 1	13,3	13,3	17,8	16,9	16,2	16,9	13,3	13,1	13,3	1,5	1,6	1,7	1,2	1,2	1,3	1,1	1,2	1,7	1,7
hedraiaandra 2	13,1	13,0	16,9	17,4	15,8	16,5	13,1	13,0	13,1	1,5	1,6	1,7	1,2	1,2	1,3	1,1	1,2	1,7	1,7
cactorum x hedraiaandra 1	16,0	16,2	16,2	15,8	19,8	19,5	13,5	16,2	16,1	1,6	1,6	1,7	1,2	1,2	1,3	1,2	1,2	1,7	1,7
cactorum x hedraiaandra 2	16,6	16,8	16,9	16,5	19,5	20,7	13,9	16,7	16,6	1,6	1,6	1,8	1,2	1,2	1,3	1,2	1,2	1,7	1,7
idaei	12,7	12,7	13,3	13,1	13,5	13,9	17,5	12,6	12,7	1,5	1,5	1,7	1,1	1,1	1,2	1,1	1,1	1,6	1,6
cactorum x nicotiana 1	16,6	16,9	13,1	13,0	16,2	16,7	12,6	30,6	29,2	2,2	2,2	2,3	1,7	1,7	1,8	1,6	1,7	14,5	14,5
cactorum x nicotiana 2	16,8	16,8	13,3	13,1	16,1	16,6	12,7	29,2	30,8	2,2	2,2	2,3	1,7	1,7	1,8	1,6	1,7	14,7	14,7
iranica	1,5	1,5	1,5	1,5	1,6	1,6	1,5	2,2	2,2	23,2	7,1	4,1	1,1	1,1	1,2	1,1	1,1	1,3	1,3
clandestina	1,5	1,5	1,6	1,6	1,6	1,6	1,5	2,2	2,2	26,5	3,8	1,1	1,1	1,1	1,2	1,1	1,1	1,3	1,3
tentaculata	1,7	1,7	1,7	1,7	1,7	1,8	1,7	2,3	2,3	4,1	3,8	23,9	1,1	1,1	1,2	1,1	1,1	1,3	1,4
mirabilis	1,2	1,2	1,2	1,2	1,2	1,2	1,1	1,7	1,7	1,1	1,1	1,1	19,4	10,7	11,9	10,6	10,6	1,0	1,0
ipomoeae	1,2	1,2	1,2	1,2	1,2	1,2	1,2	1,7	1,7	1,1	1,1	1,1	10,7	19,9	11,5	10,3	10,3	1,0	1,0
andina (infestans hybrid)	1,2	1,2	1,3	1,3	1,3	1,3	1,2	1,8	1,8	1,2	1,2	1,2	11,9	11,5	23,6	16,1	16,2	1,1	1,1
infestans 1	1,1	1,1	1,1	1,1	1,2	1,2	1,1	1,6	1,6	1,1	1,1	1,1	10,6	10,3	16,1	19,7	17,6	1,0	1,0
infestans 2	1,1	1,1	1,2	1,2	1,2	1,2	1,1	1,7	1,7	1,1	1,1	1,1	10,6	10,3	16,2	17,6	19,9	1,0	1,0
nicotiana 1	1,7	1,7	1,7	1,7	1,7	1,7	1,6	14,5	14,7	1,3	1,3	1,3	1,0	1,0	1,1	1,0	1,0	16,1	14,9
nicotiana 2	1,7	1,7	1,7	1,7	1,7	1,7	1,6	14,5	14,7	1,3	1,3	1,4	1,0	1,0	1,1	1,0	1,0	14,9	16,2

- hybrids (blue) share large number of loci with at least two different species (>80% loci in common)
- the more phylogenetically distinct the parental species are, the more loci the (sexual) hybrids will have (cfr cactorum x hedraiaandra vs cactorum x nicotiana)

SNP-based similarity between common loci (%)

	cactorum 1	cactorum 2	hedraiaandra 1	hedraiaandra 2	cactorum x hedraiaandra 1	cactorum x hedraiaandra 2	idaei	cactorum x nicotiana 1	cactorum x nicotiana 2	iranica	clandestina	tentaculata	mirabilis	ipomoeae	andina (infestans hybrid)	infestans 1	infestans 2	nicotiana 1	nicotiana 2
cactorum 1	100	88	40	40	89	89	35	89	88	1	1	1	1	1	1	1	1	1	1
cactorum 2	88	100	40	40	98	98	34	99	90	1	1	1	1	1	1	1	1	1	1
hedraiaandra 1	40	40	100	88	93	96	38	40	40	1	1	1	1	1	1	1	1	1	1
hedraiaandra 2	40	40	88	100	81	83	38	39	39	1	1	1	1	1	1	1	1	1	1
cactorum x hedraiaandra 1	89	98	94	83	100	99	44	95	87	1	1	1	1	1	1	1	1	1	1
cactorum x hedraiaandra 2	89	98	96	85	99	100	44	95	87	1	1	1	1	1	1	1	1	1	1
idaei	35	34	38	38	44	44	100	34	34	0	1	0	1	1	1	1	1	1	1
cactorum x nicotiana 1	89	99	40	39	95	95	34	100	86	1	1	1	1	1	1	1	1	86	86
cactorum x nicotiana 2	88	90	40	39	87	87	34	86	100	1	1	1	1	1	1	1	1	88	87
iranica	1	1	1	1	1	1	0	1	1	100	12	3	1	1	1	1	1	1	0
clandestina	1	1	1	1	1	1	1	1	1	12	100	2	1	1	1	1	1	1	1
tentaculata	1	1	1	1	1	1	0	1	1	3	2	100	1	1	1	1	1	1	1
mirabilis	1	1	1	1	1	1	1	1	1	1	1	1	100	26	33	27	28	1	1
ipomoeae	1	1	1	1	1	1	1	1	1	1	1	1	27	100	32	25	26	1	1
andina (infestans hybrid)	1	1	1	1	1	1	1	1	1	1	1	1	33	32	100	80	80	1	1
infestans 1	1	1	1	1	1	1	1	1	1	1	1	1	28	25	80	100	94	1	1
infestans 2	1	1	1	1	1	1	1	1	1	1	1	1	28	25	80	94	100	1	1
nicotiana 1	1	1	1	1	1	1	1	86	88	1	1	1	1	1	1	1	1	100	89
nicotiana 2	1	1	1	1	1	1	1	86	87	0	1	1	1	1	1	1	1	89	100

- different genotypes can be distinguished within species
- parental genotype of a hybrid can be determined (e.g. cactorum 2 has the parental genotype of both the cactorum x hedraiaandra and the cactorum x nicotiana 1 hybrids)

Conclusions

- unknown isolates can undoubtedly be identified to species level
- species that are assumed to be non-hybrids contain between ~12000 to ~35000 GBS loci (average ~22000 loci)
- allopolyploid hybrids such as *P. xalni* contain an extremely large number of GBS loci (>45000)
- hybrids can be distinguished from non-hybrid species:
 - hybrids have >80% of loci in common with at least two different species
 - hybrids contain more loci than both the parental species
- the SNP data:
 - can distinguish between different genotypes
 - can elucidate the parental genotype of a hybrid
- inclusion of reference species in the analysis is crucial for correct identification and for evaluation of the hybrid status of unknown isolates