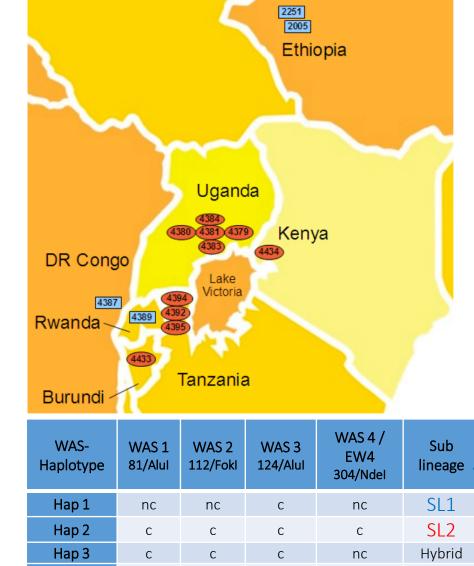
# IPME A MLVA scheme for the analysis of population genetic diversity and structure of Xanthomonas vasicola pv. musacearum

### Why do we need a MLVA scheme for XVM ?

- *Xanthomonas vasicola* pv. *musacearum* (Xvm) is a genetically monomorphic bacterial pathogen
- Several questions remain about the ecology, evolution, population genetic diversity of this major *Musaceae* pathogen
- The only genotyping tools available to date are Wasukira's **SNP-derived RFLP markers.**
- There is a strong need for highly polymorphic markers.
- We describe here the development of a set of 19 VNTR



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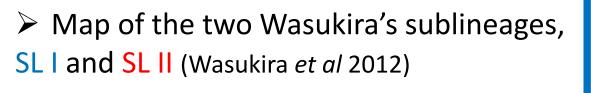
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Primer 2R

#### Valentine NAKATO<sup>1</sup>, Juan Luis FUENTES ROJAS<sup>2</sup>, Christian VERNIERE<sup>3</sup>, Laurence BLONDIN<sup>3</sup>, George MAHUKU<sup>4</sup>, Teresa COUTINHO<sup>5</sup>, Emmanuel WICKER<sup>2,6</sup>

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<sup>1</sup>IITA, Kampala, Uganda ; <sup>2</sup>Univ. Montpellier-CIRAD, UMR IPME, Montpellier, France ; <sup>3</sup>CIRAD, UMR BGPI, Montpellier, France; <sup>4</sup>CIRAD, UMR IPME, Montpellier, France ; <sup>4</sup>IITA, Dar Es Salaam, Tanzania <sup>5</sup>University of Pretoria, Pretoria, South Africa; <sup>6</sup>CIRAD, UMR IPME, F - 34398 Montpellier, France

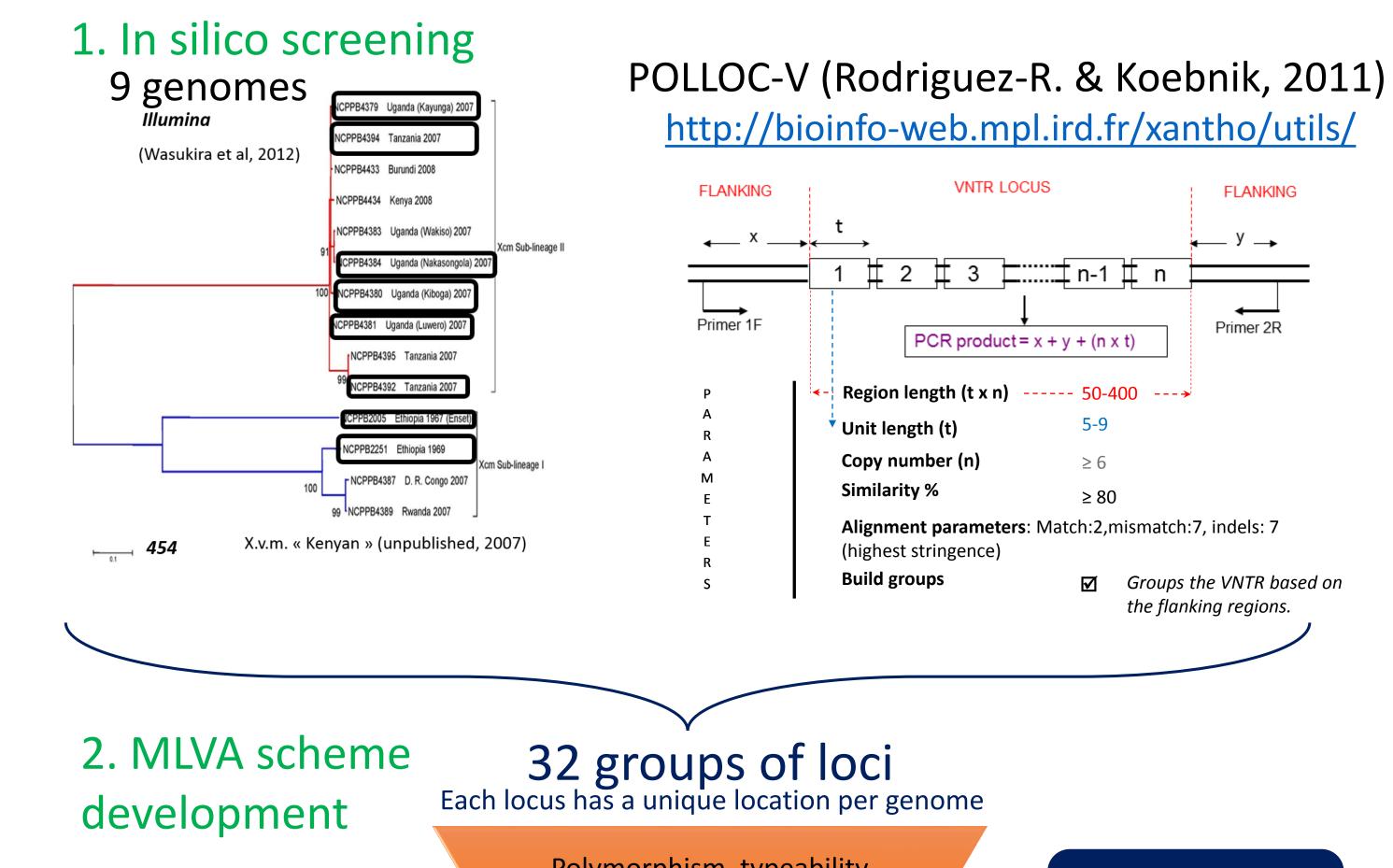


> The Wasukira's SNP-derived markers (Wasukira *et al* 2012)

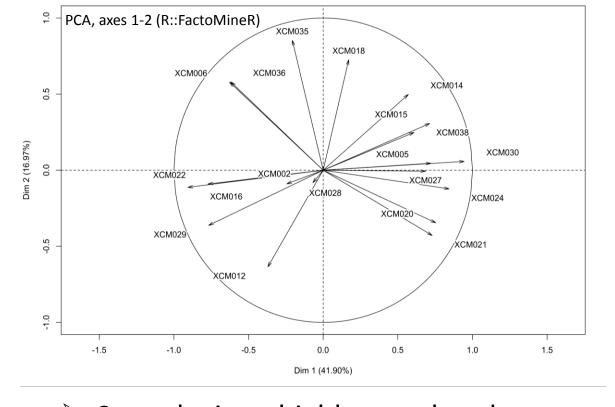
markers, fully adapted to address molecular epidemiology questions.

Hybrid nc c: cut; nc: not cut

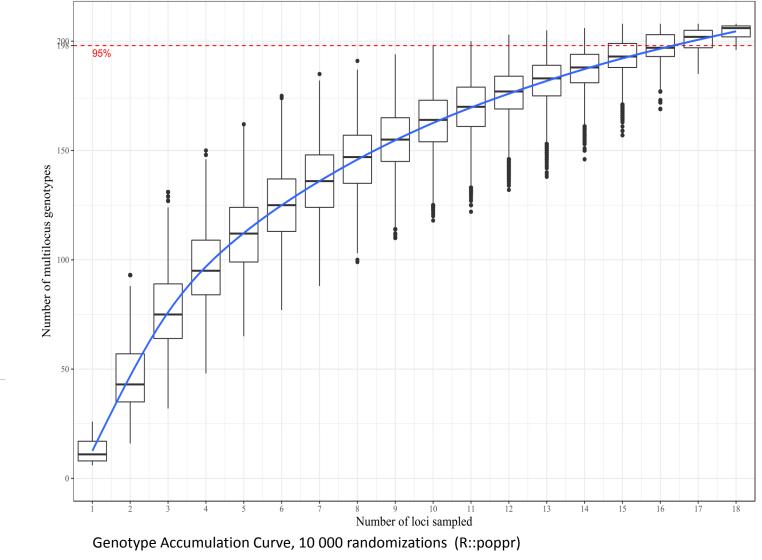
#### The approach



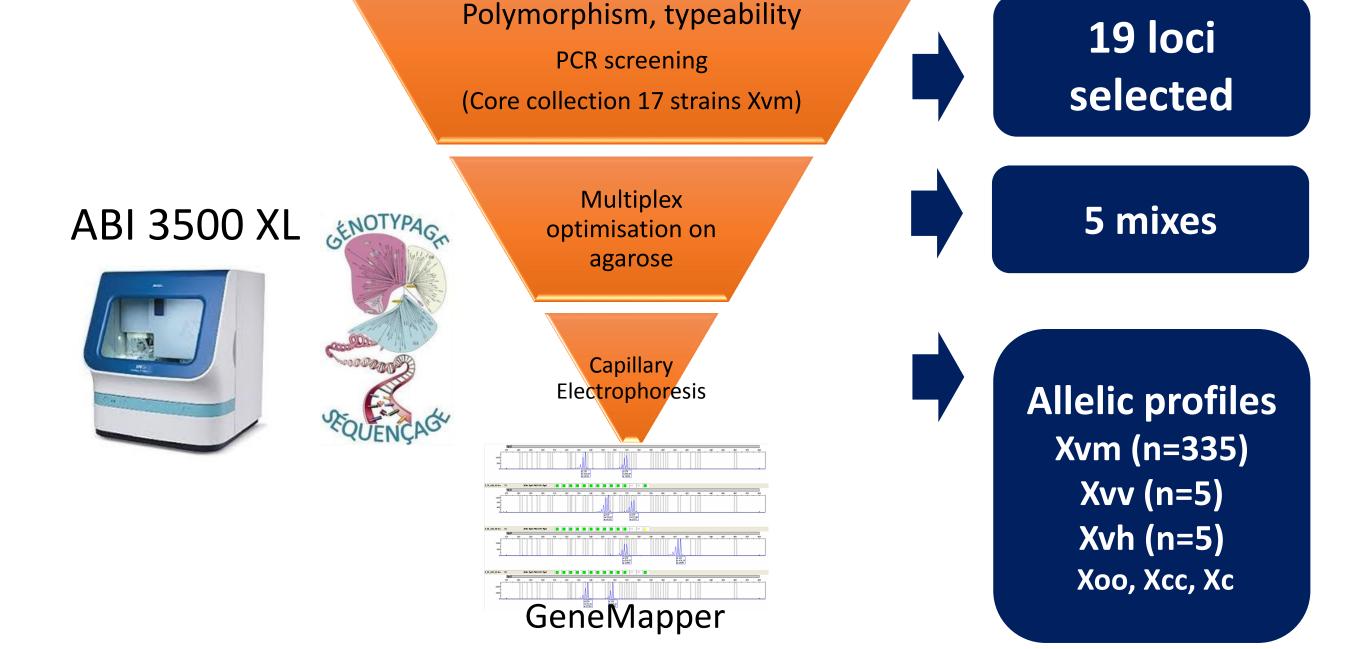
#### 3. Is MLVA-19 resolutive enough ?



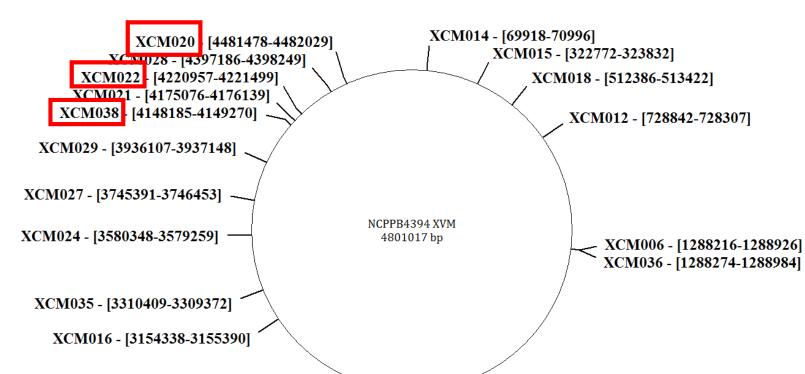
- Some loci are highly correlated ➤ XCM006/XCM036
- ➤ XCM016/XCM022
- Contributions of XCM028 and XCM002 to the 2-axes are low
- > 13 directions
  - > are 13 loci sufficient to determine the genetic diversity and structure of Xvm?



- 95% of the MLG detected with 16 loci
- 19 loci are sufficient to detect most of the genotypic diversity
- Decrease the number of loci would mitigate the resolutive power



### 1. MLVA-19, the first MLVA scheme for Xanthomonas vasicola



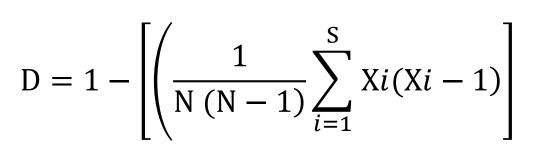
- All but 3 loci are intergene (intragene: Hyp. Protein)
- Mostly microsatellites (6-9 bp), a majority of 7 bp repetitions
- Number of alleles per locus on the 335 strain collection:

• Min = 6 (XCM018)

• Max = 25 (XCM027)

# 4. MLVA-19 is more discriminatory than existing SNP-derived markers

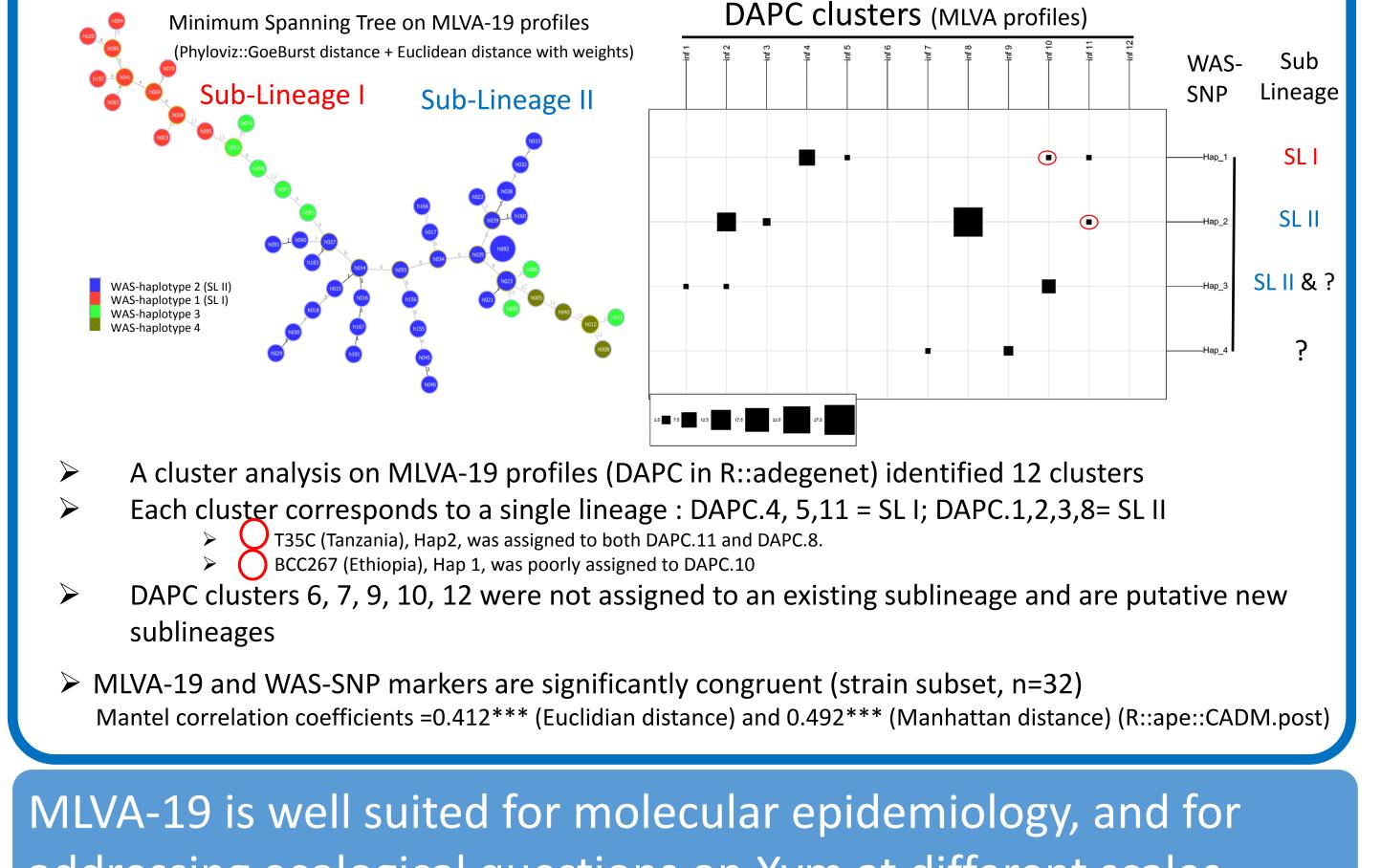
Hunter-Gaston Discriminatory Index (HGDI)

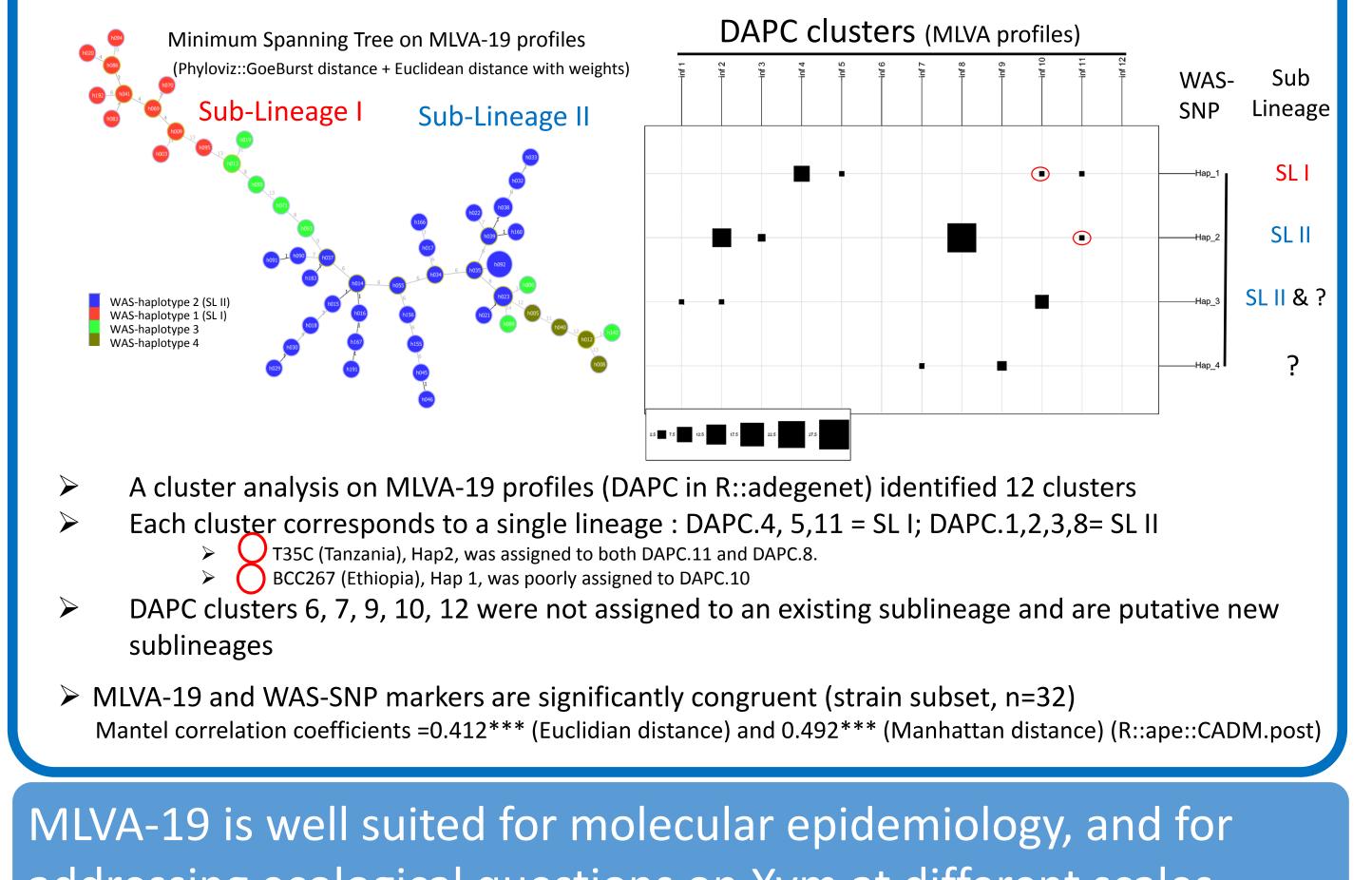


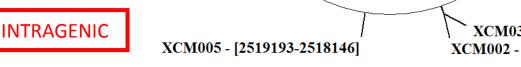
N: total number of isolates S : total number of types Xi: number of isolates belonging to the *i*th type WAS-SNPs: 4 SNP-derived RFLP loci (Wasukira et al 2012) **VN-SNPs**: 4 additional SNP-derived RFLP loci (V. NAKATO, 2017 unpublished)

	Total collection (n=63)		Core collection (n=32)		
Typing technique	No. of haplotypes	HGDI	No. of haplotypes	HGDI	
MLVA	63	0.981	32	1.000	
WAS-SNPs	4	0.555	4	0.754	
VN-SNPs	-	-	5	0.740	

# 5. MLVA-19 is consistent with whole-genome phylegeny, and reveals unexpected diversity







XCM030 - [2151016-2151719] XCM002 - [2152939-2152217]

#### 2. A highly typeable and specific scheme

- **Typeability on Xvm**: 18 loci amplify more than 97% strains
- Specificity
  - > XCM012 is Xvm-specific
  - Most loci amplify Xvm and Xvv
  - > Xvm-specific loci

Loci specificity further confirmed that Xcm is a <u>X. vasicola</u> clade

Locus	X.vasicola pv.			X. oryzae	X.campestris	X. citri
	musacearum	vasculorum	holcicola	pv. <i>oryzae</i>	pv. cannabis	
XCM002	100	40	60	0		
XCM005	100	40	0	0		
XCM006	97.6	20	60	<mark>5</mark> 0		
XCM012	97.9	0	0	0		
XCM014	99.1	60	20	0		
XCM015	90.4	100	0	0		
XCM016	99.1	80	40	0		
XCM018	99.4	0	20	0		
XCM020	99.4	60	40	0		
XCM021	99.1	40	20	0		
XCM022	99.7	100	60	0		
XCM024	100	100	0	0		
XCM027	99.1	60	20	0		
XCM028	100	60	40	0		
XCM029	99.4	100	60	0		
XCM030	99.7	80	40	0		
XCM035	99.1	80	0	0		
XCM036	97.3	40	40	<mark>5</mark> 0		
XCM038	99.4	60	0	0		
Total	335	5	5	2	1	1

Proportion of amplified isolates within each pathovar/species



#### Acknowledgements

David STUDHOLME, Ralf KOEBNIK

GenSeq technical facilities of the « Institut des Sciences de l'Evolution de Montpellier » - Labex "Centre Méditerranéen de l'Environnement et de la Biodiversité" (Mediterranean Center for Environment and Biodiversity).

addressing ecological questions on Xvm at different scales, from the country to the field. See the presentation of V. NAKATO, Session V, Saturday July 21.