

A large scale effort to analyse the *Plum pox virus* diversity worldwide.

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SAVBA Bratislava (**Slovakia**), FRI Cacak (**Serbia**), SCDP Bistrita (**Romania**)





WPE1 tasks

Large scale analysis of PPV diversity through the partial sequencing (target: ca. 700 isolates from the world)

Complete genomic characterization of selected, epidemiologically relevant PPV isolates (target: ca. 30 isolates)

Development of long term reference collections and data integration

Development of technology for the rapid genome-wide analysis of PPV isolates

12 laboratories from 10 partner countries + collaborators



Objectives

Generate a global picture of the currently spreading *Plum pox virus* isolates diversity, not only within the participating countries but at the world level.

Contribute to the understanding of the geographic dynamics and the genetic evolution of the PPV population

Develop innovative technologies for the rapid and detailed characterization of PPV isolates

Further applications for the development of a risk management and early warning system



Start point

A number of PPV sequences in the public database, but limitations for large-scale analysis of the genetic diversity

- strain- and geographical disproportion and bias
- lack of traceability in many cases (sequence, but no data about isolate)
- isolates with propagation history in herbaceous hosts...

7 recognised PPV strains

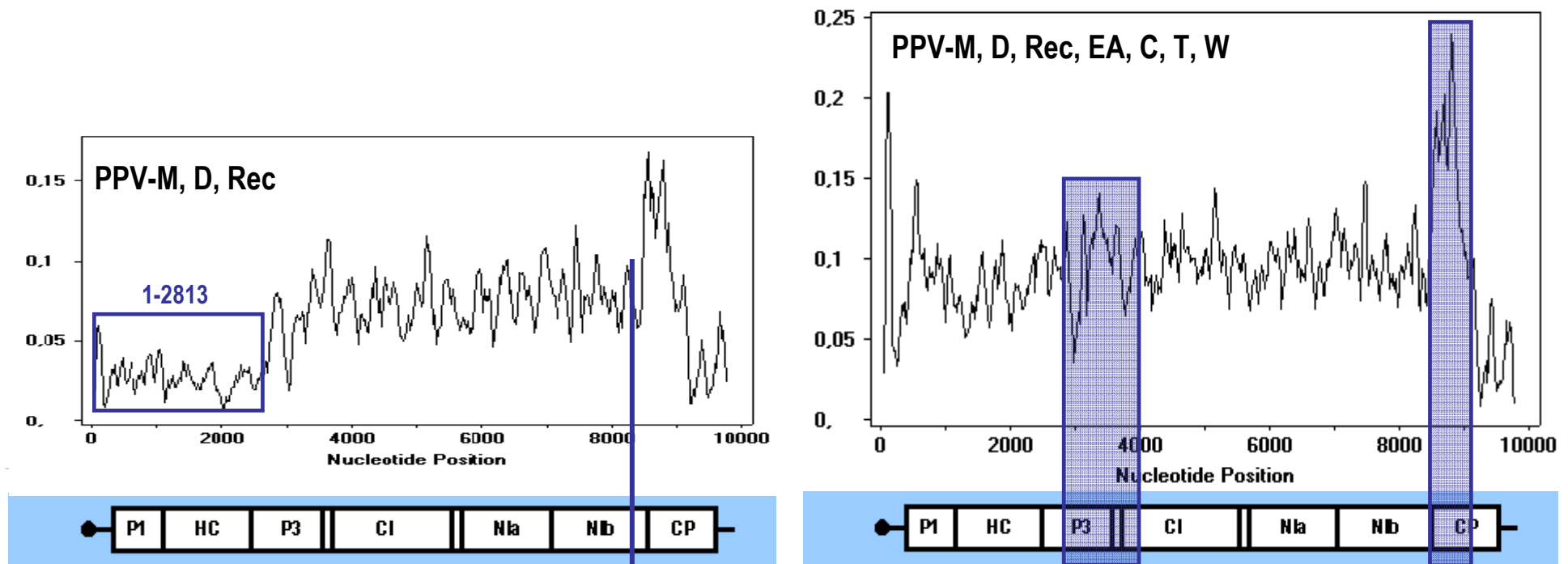
M, **D** (early 1990's), **EA** (1991), **C** (1996),
Rec (2001), **W** (2003), **T** (2009)

The two most informative genomic regions were selected for partial sequencing

N-ter CP (450 bp)

P3-6K1 (830 bp)

average nucleotide diversity

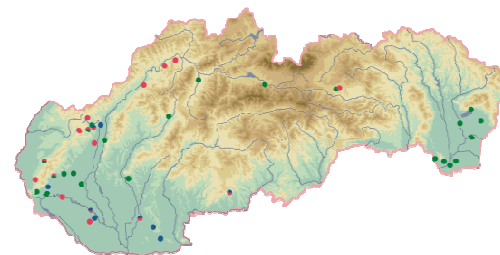


- recombination breakpoint in PPV-Rec is situated at the end of the NIb gene
- PPV-D, PPV-M and PPV-Rec isolates share an ancestrally common 5' region (5' NCR - N-ter P3) so that P1-HC is poorly informative for these strains



A common protocol used

- sampling reflecting regional particularities (geography, hosts, PPV diversity ...)



- labelling of the tree, epidemiological data recorded (isolate sheet)

- standardised RT-PCR protocol
- direct sequencing of PCR products to obtain dominant sequence
- lyophilisation of original infected *Prunus* material (or grafting for *in vivo* collection)
- integration of data (sequence, isolate information) to the SharCo database



Some statistics

In August 2010 ca. **530**
isolates from 20 countries
analysed
already 2x > than NCBI

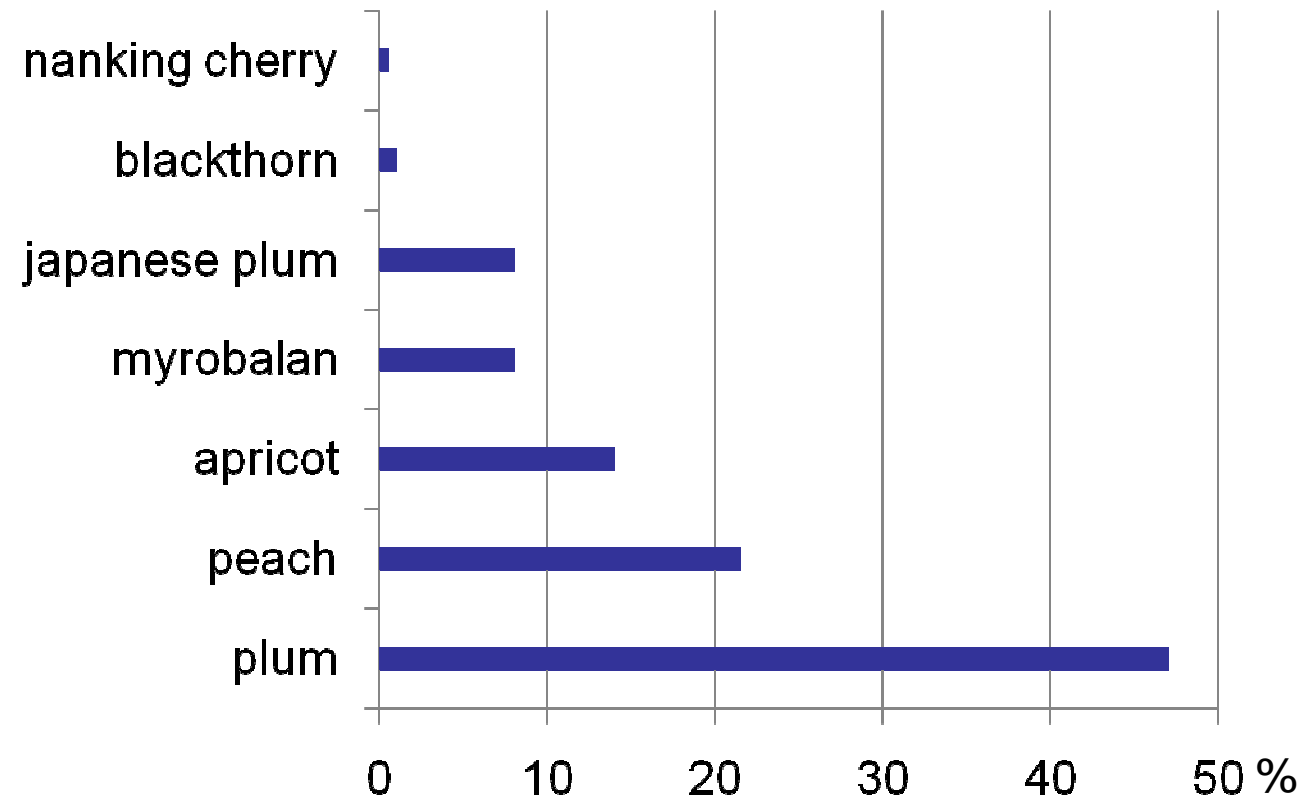
6 out of 7 PPV strains
detected and
characterised (except EA)

D	47%
M	33%
Rec	14%
T	5%
C, W	1%

Albania	2
Austria	1
Belarus	13
Bulgaria	52
Czech Republic	52
France	63
Germany	12
Italy	28
Latvia	13
Lithuania	2
Macedonia	7
Moldova	12
Norway	2
Poland	31
Romania	36
Saudi Arabia	1
Serbia	55
Slovakia	58
Spain	41
Turkey	48



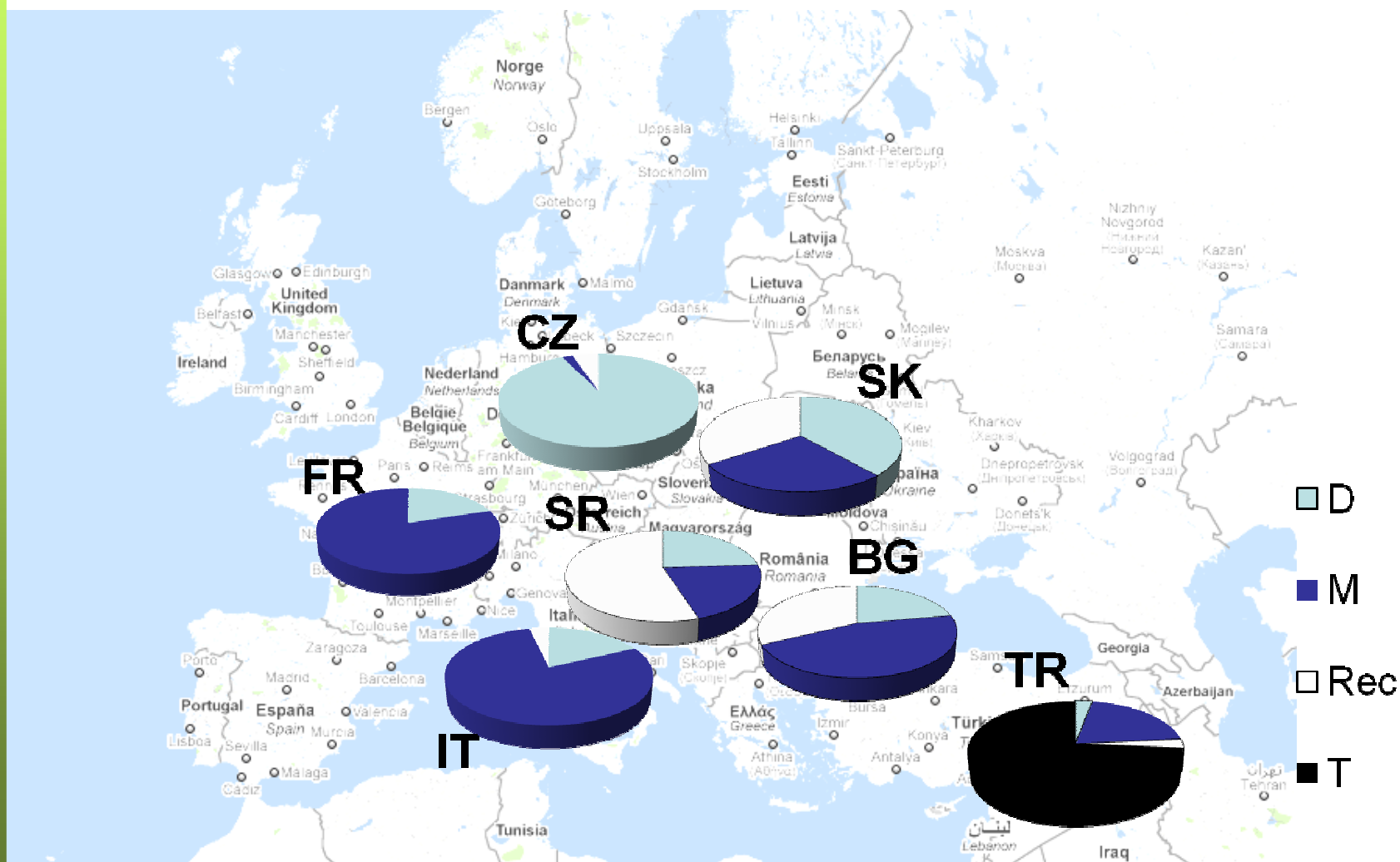
PPV isolates by the original host:



Limited previous information from some of these hosts



Representations of PPV strains in some partner SharCo countries

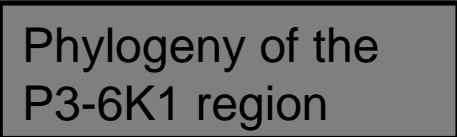
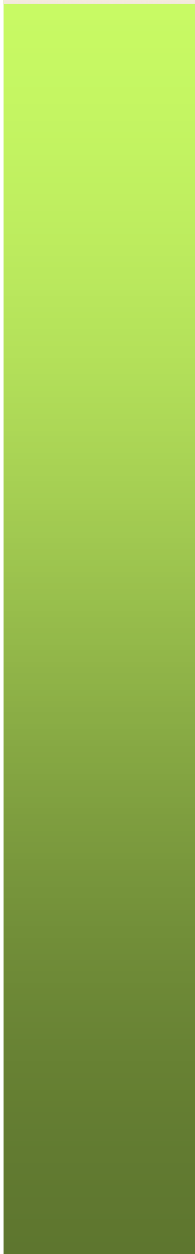


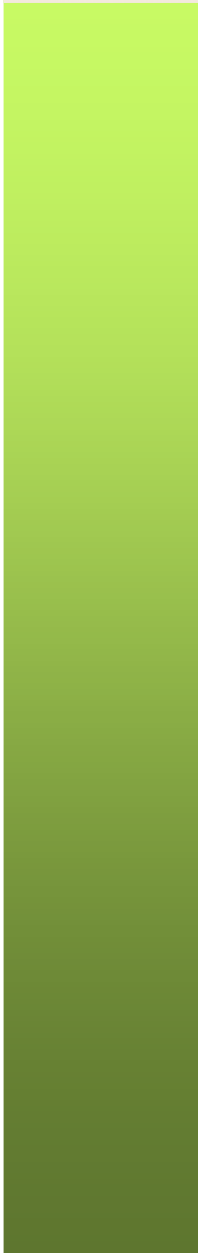


A host preference should be noted for major PPV strains

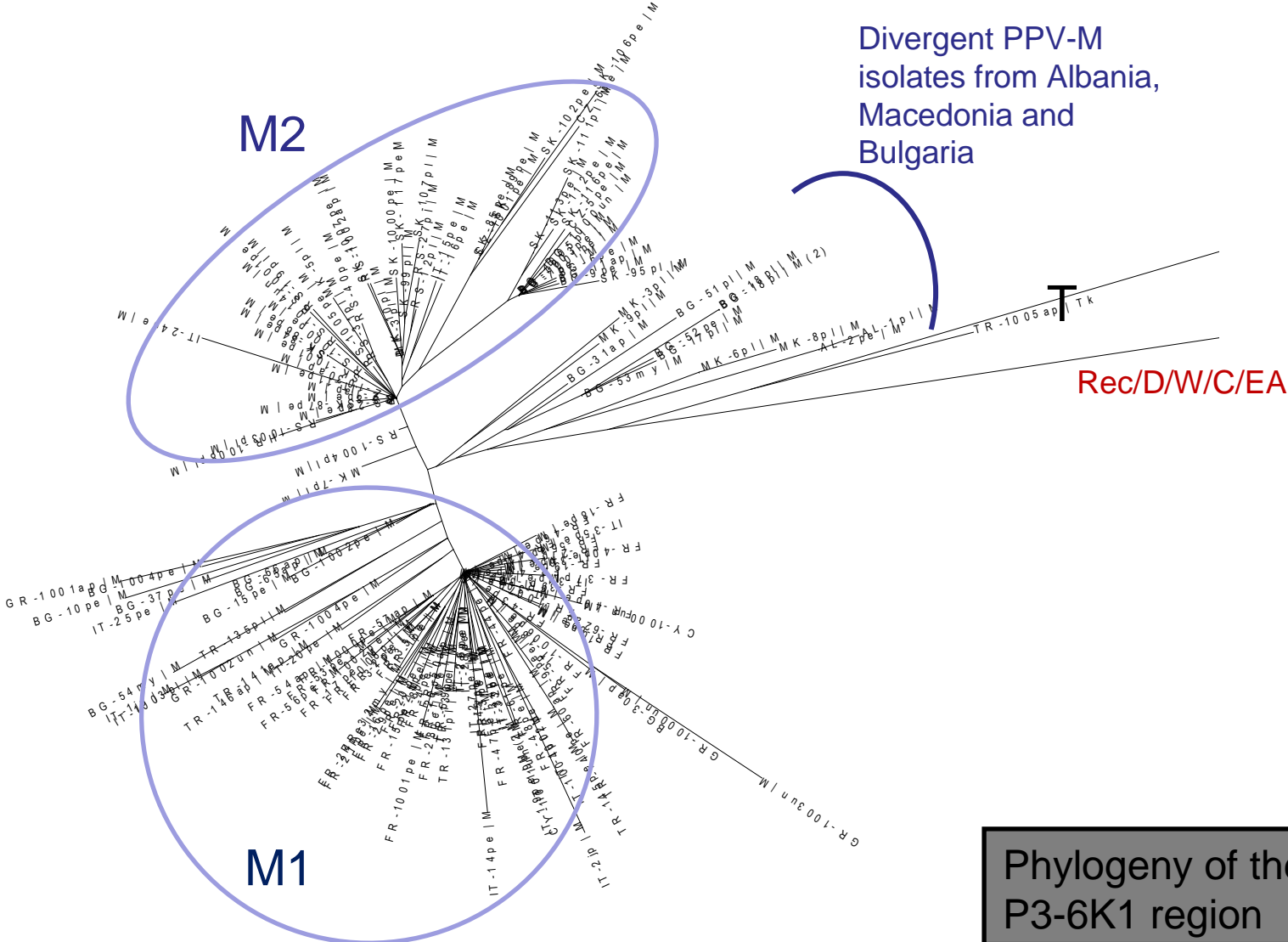
- peaches predominantly infected by PPV-M, myrobalans by PPV-D
- a rare infection of peaches by PPV-Rec

	D (%)	M (%)	Rec (%)	T (%)
<i>Prunus armeniaca</i> (Apricot)	46,1	27,7	4,6	21,5
<i>Prunus cerasifera</i> (Myrobalan)	78,8	9,1	12,1	
<i>Prunus domestica</i> (Plum)	57,4	12,4	29,2	1,0
<i>Prunus persica</i> (Peach)	9,9	87,1	1,0	2,0



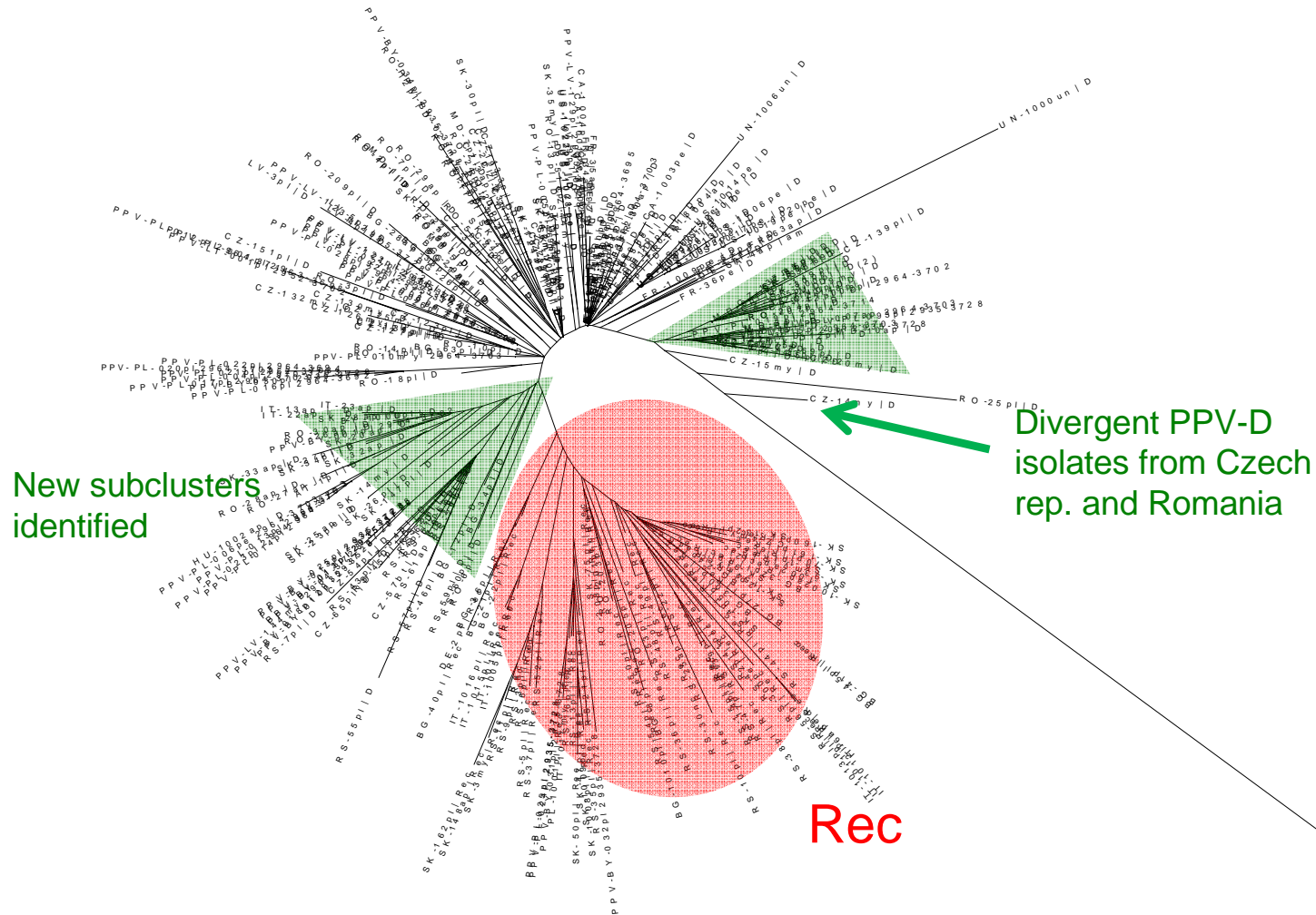


Two major clusters of PPV-M isolates but more diversity identified



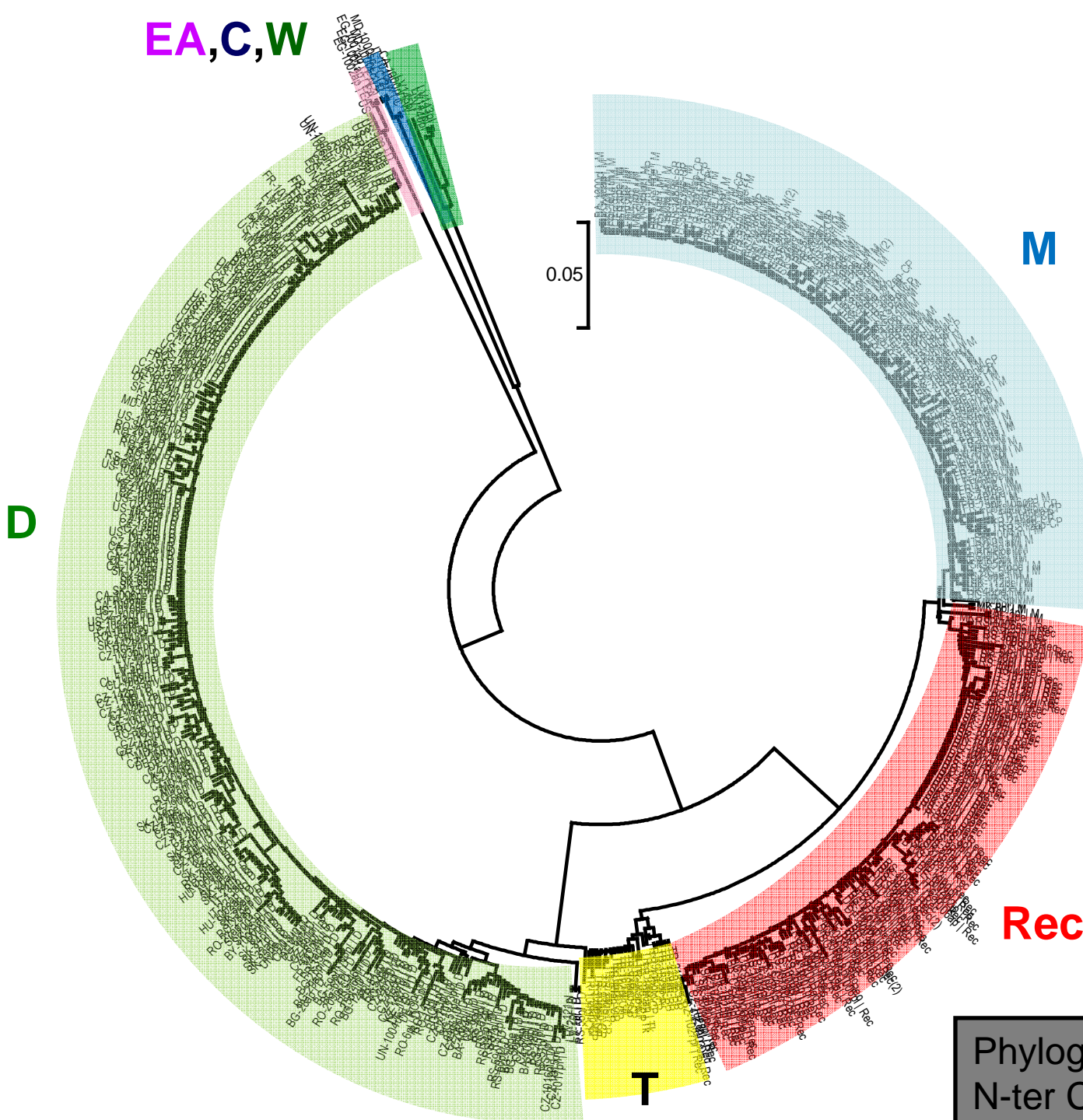


Diversity within PPV-D is higher than previously known



PPV-Rec isolates phylogenetically distinct from PPV-D

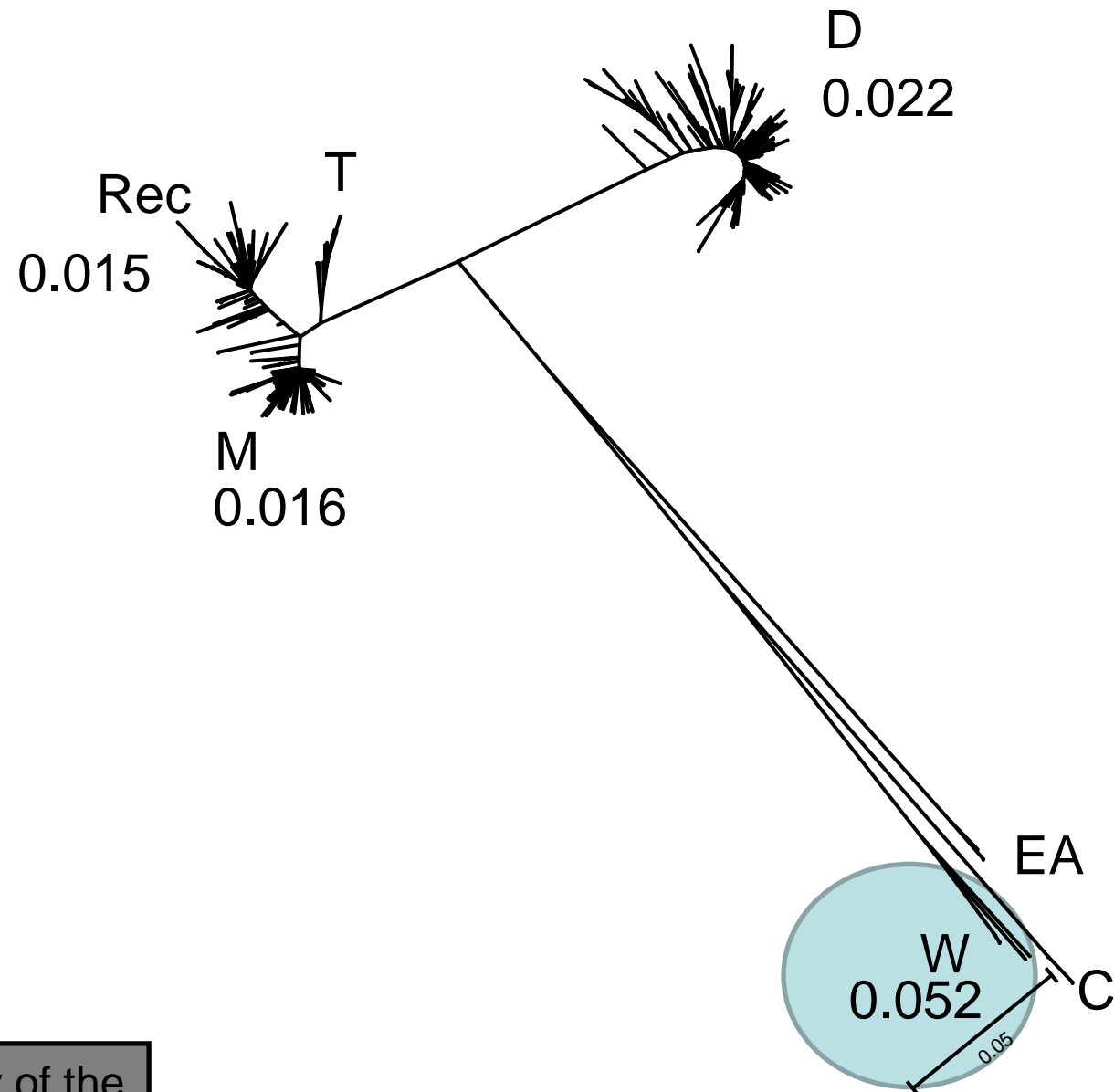
Phylogeny of the P3-6K1 region



Phylogeny of the
N-ter CP region



Within-group genetic diversity



Phylogeny of the
N-ter CP region



Conclusions (not yet definite):

- Higher than previously envisioned intra-strain variability observed within PPV-D: identification of divergent isolates, mostly from central- and eastern Europe.
- Confirmation of the splitting of the PPV-M strain into two groups, existence of divergent isolates.
- High prevalence of isolates belonging to the newly discovered PPV-T strain in Turkey.



Conclusions (not yet definite):

- New information on distribution of PPV strains (presence of PPV-Rec in Italy, PPV-W in Latvia, PPV-C in Belarus)
- „Divergent“ isolates identified in each strain as potential targets for full genome sequencing

Tack

Vielen Dank

Obrigado

Paldies

Merci

ありがとうございます

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Grazie

ขอบคุณ

Спасибо

Thank You

Kiitos

Tak

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감사합니다

Gracias

Σας ευχαριστούμε

Dziękujemy

