High throughput sequencing (HTS) provides new insights into the olive leaf yellowing complex

EPPO Workshop for Heads of Plant Pest Diagnostic Laboratories
Saku (EE), 2025-03-06/07

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- ✓ **Olive is a strategic crop in Spain**
- ✓ **Spain main producer with 2,651,110 ha cultivated**
- ✓ **Production 5,101,010 tones in 2023 (FAOSTAT)**

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ECOLIVE

Epidemiology and control of olive leaf yellowing syndrome and emerging viruses in olive in Spain PID2021-128779OR-100

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Olive leaf yellowing syndrome

- **Vein yellowing**

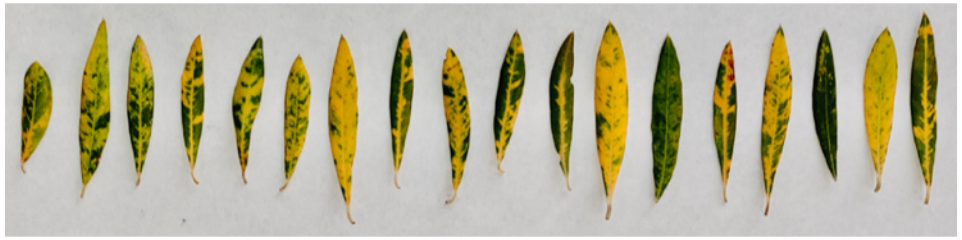
  OVYaV, putative potexvirus, no genomic information available

- **Leaf yellowing**

  OLYaV, unassigned closterovirus, only partial genomic information available

- **Yellow mottling and decline**

  OYMDaV, putative capillovirus or trichovirus, no genomic information available



Olive leaf yellowing associated virus (OLYaV)

HTS analysis

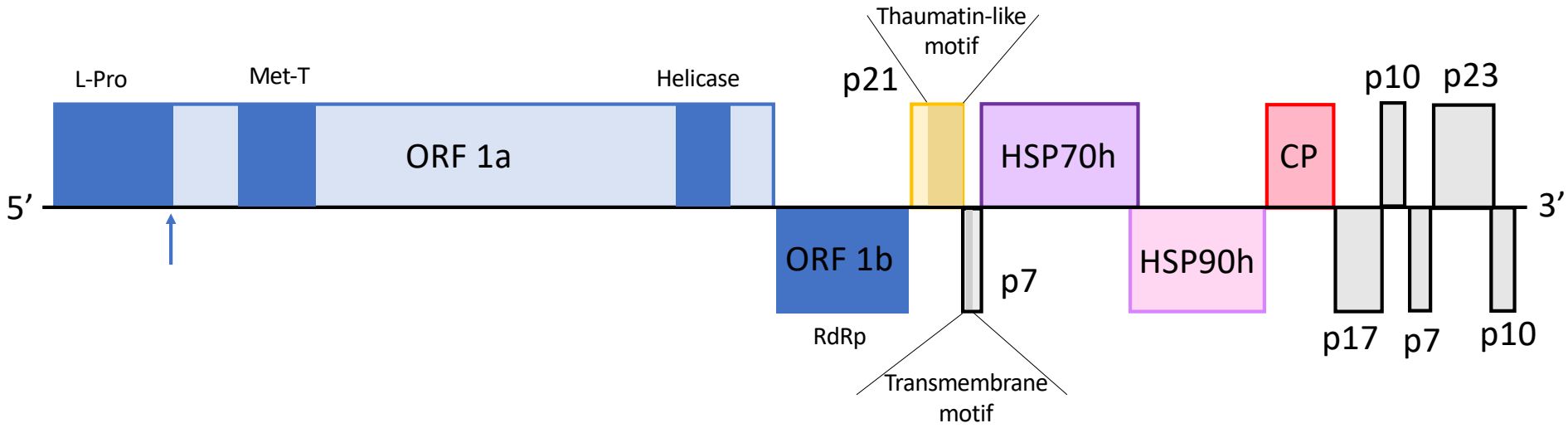
Leaf yellowing and wood deformation
CS1, Brazil

Leaf yellowing and intense defoliation
V64, Spain

Olive leaf yellowing associated virus (OLYaV)

Family *Closteroviridae*, genus *Olivavirus*

Olivavirus flavioleae



Ruiz-García *et al*., 2020; Ruiz-García *et al*., 2021

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Olive leaf yellowing associated virus (OLYaV)

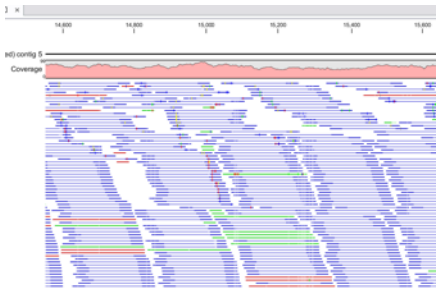
- ❑ Study OLYaV genetic diversity
- ❑ Develop inclusive detection methods
- ❑ Identify transmission vectors

OLYaV genetic diversity HTS

Total RNA purification

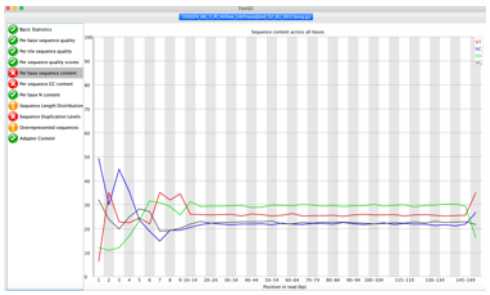
cDNA synthesis and library preparation

Illumina HTS paired-end (150pb)



Contig extension

De novo assembly

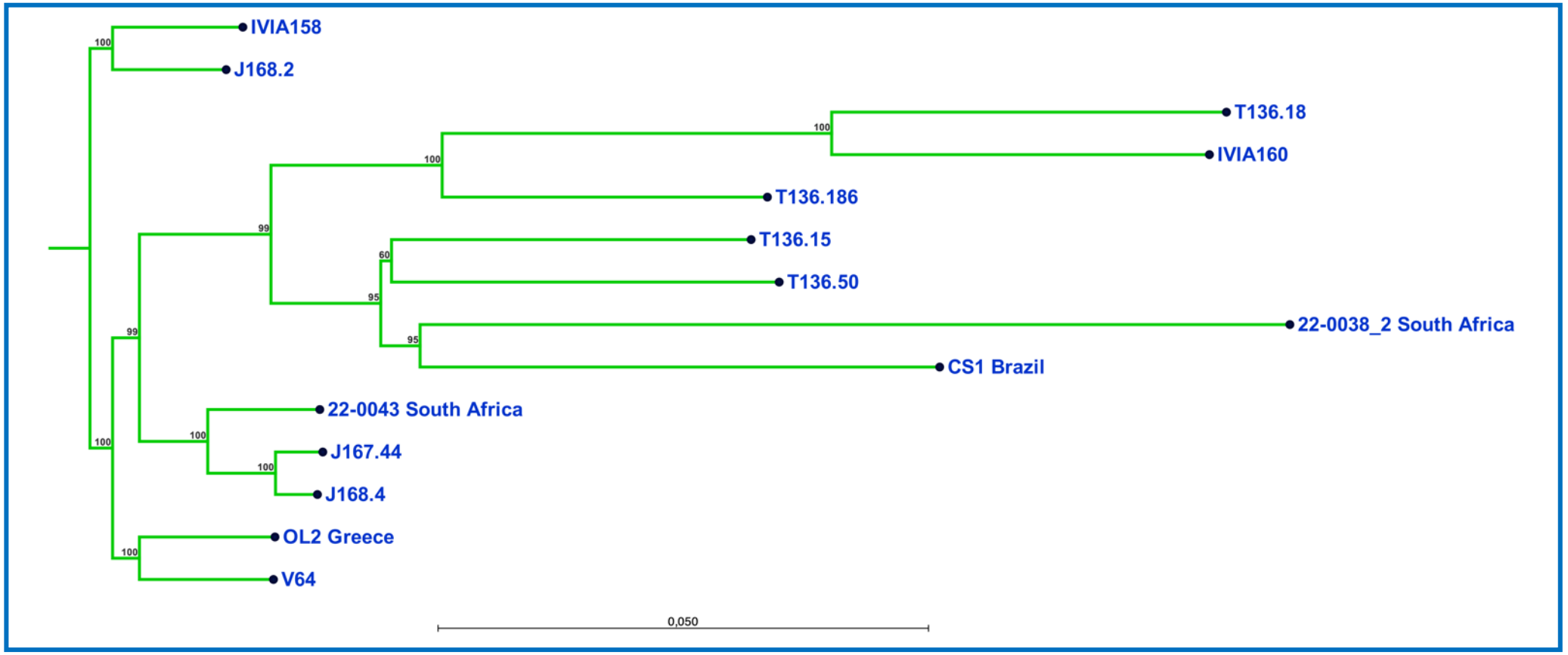


Trimming and QC

OLYaV genetic diversity

	Isolate	Origin	Cultivar	Length (bp)	Accession number
●	T136.15	Tarragona	Manzanilla	16,658	PQ295886
●	IVIA158	Valencia	Olaya	16,725	PP855214
●	J168.2	Jaén	Picual	16,724	PP855216
●	J167.44	Jaén	Picual	16,721	PP855724
●	J168.4	Jaén	Picual	16,725	PP855725
●	T136.50	Tarragona	Manzanilla	16,703	PQ295888
●	T136.186	Tarragona	Manzanilla	16,700	PQ255887
●	T136.18	Tarragona	Manzanilla	16,699	PQ295889
●	IVIA160	Valencia	Olaya	16,704	PP855215

OLYaV genetic diversity



OLYaV genetic diversity: diagnostic implications

Isolate	5'UTR	ORF1a	ORF1b	ORF2	ORF3	ORF4	ORF5	ORF6	ORF7	ORF8	ORF9	ORF10	ORF11	3'UTR
T136.15	98.0	90.5	91.3	92.1	92.1	91.9	91.7	90.5	93.1	90.3	87.2	91.4	95.7	99.4
OL2	99.0	96.0	97.1	94.0	94.7	97.1	96.1	97.4	96.9	97.5	95.9	97.2	97.8	100
IVIA158	100	94.7	97.0	94.6	94.2	95.6	94.4	97.0	96.9	95.3	92.3	96.1	97.5	95.5
J168.2	100	95.0	97.2	94.7	95.2	95.4	95.0	96.9	97.1	97.5	92.8	96.6	98.6	100
J167.44	99.0	94.5	96.5	94.6	96.3	95.2	95.1	94.7	97.1	96.4	90.3	96.1	97.1	98.1
J168.4	100	94.3	96.1	94.7	96.3	95.4	95.7	94.9	97.3	96.4	91.3	96.6	97.1	100
T136.50	98.0	89.9	92.8	91.6	95.8	91.3	91.3	91.7	92.7	89.6	88.7	87.7	90.2	97.4
T136.186	98.0	81.2	96.7	94.4	95.2	95.4	95.5	95.7	96.0	95.7	94.9	95.6	96.0	96.1
CS1	99.0	91.0	92.7	89.5	93.7	79.8	79.6	85.5	81.3	81.0	78.5	84.3	89.5	96.8
T136.18	96.1	81.2	84.7	80.7	82.5	79.5	79.6	86.8	82.5	80.3	77.4	83.1	90.9	97.4
IVIA160	96.1	80.4	84.6	83.9	85.2	80.4	80.1	86.8	81.3	83.2	76.9	91.3	97.5	94.9
Average	97.4	87.8	91.3	89.6	90.8	87.1	88.0	90.8	90.0	88.2	85.3	89.9	93.8	96.8

98 – 100 % 95 – 98 % 90 – 95 % 80 – 90 % < 80 %

Study on OLYaV transmission

OLYaV testing on insects collected from infected trees:

- Family *Cicadellidae*
- Family *Aphididae*
- *Agalmatium flavescens* (family *Issidae)*
- *Tingissus tangirus* (family *Issidae)*
- *Bergevinium angulare* (family *Issidae)*
- *Euphyllura olivina* (family *Psyllidae)*

Only *Euphyllura olivina* tested positive for OLYaV

OLYaV transmission experiments

Acquisition 2-4 weeks

Transmission on going…

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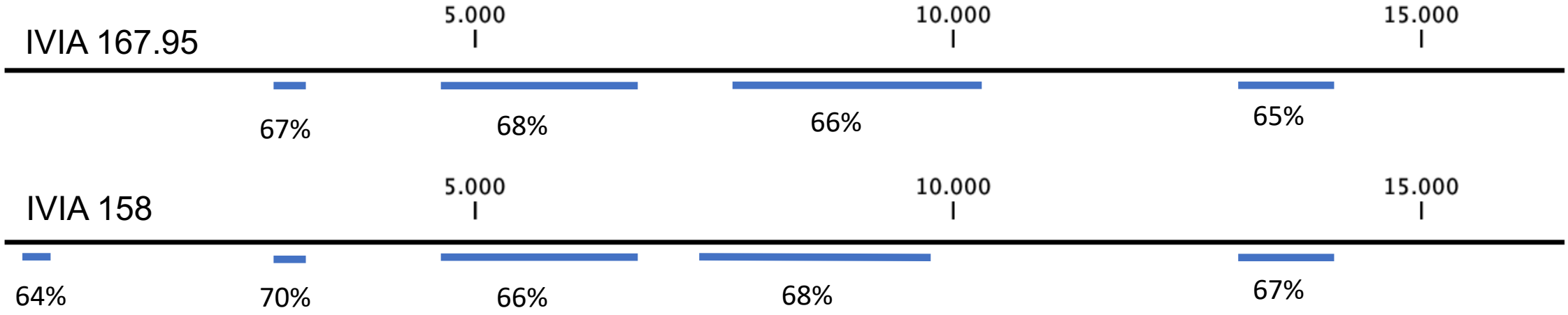
Identification of a new olivavirus

IVIA 158, Germplasm collection of traditional varieties, Valencia, cv. Olaya

Identification of a new olivavirus

IVIA 167.95, Jaén, cv. Picual

HTS analysis

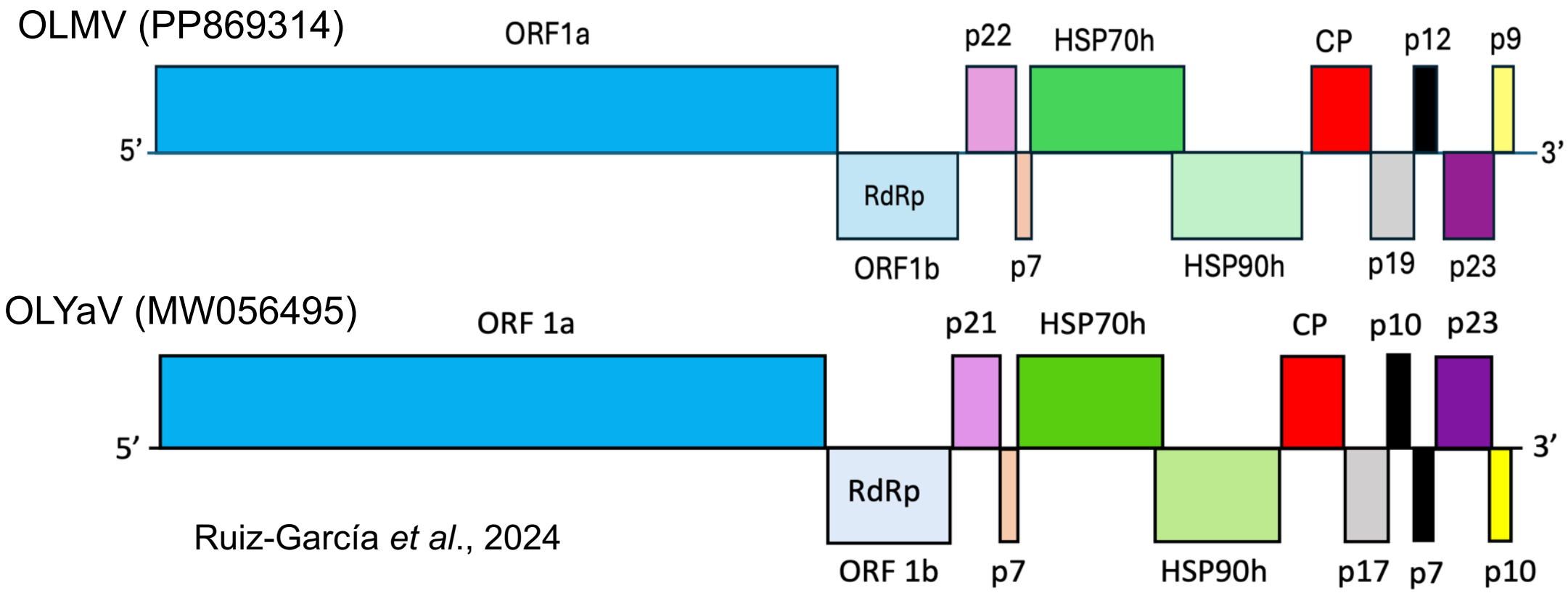


Isolate	Length (nt)	Nucleotide identity OLYaV (V64)
IVIA 167.95	16,516	68 % (45 % coverage)
IVIA 158	16,489	67 % (43 % coverage)

IVIA 167.95 and IVIA 158 shared 86.8% nt identity

Olive leaf mottling virus (OLMV)

A new member of the genus *Olivavirus*



Ruiz-García *et al*., 2024

Olive leaf mottling virus (OLMV)

A new member of the genus *Olivavirus*

Virus	Isolate	Origin	ORF1a	RdRp	Thaumatin-like protein	HSP70h	HSP90h	CP
OLYaV	CS1 (MT809205)	Brazil	94.58%	98.61%	89.47%	88.79%	87.02%	97.86%
	OL2 (OK569886)	Greece	96.26%	98.21%	94.21%	97.79%	96.69%	97.44%
	IVIA158 (PP855214)	Spain	95.79%	98.20%	93.68%	96.94%	95.93%	99.57%
	IVIA 160 (PP855215)	Spain	87.80%	96.61%	87.89%	89.64%	87.02%	97.44%
	J168.2 (PP855216)	Spain	95.93%	98.61%	94.74%	97.28%	95.93%	98.29%
	J167.44 (PP855724)	Spain	95.68%	97.41%	93.68%	96.94%	96.71%	96.58%
	J168.4 (PP855725)	Spain	95.71%	97.22%	94.21%	97.11%	97.09%	97.01%
OLMV	**OLMV-158 (PP869314)**	Spain	**44.96%**	**71.99%**	**58.97%**	**61.97%**	**55.43%**	**60.34%**
	OLMV-167.95 (PP928841)	Spain	**45.73%**	**72.20%**	**59.49%**	**61.46%**	**56.40%**	**60.76%**

Ruiz-García *et al*., 2024

OLYaV and OLMV co-infections

OLMV +
OLYaV +

OLMV +
OLYaV -

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Conclusions

- **OLYaV complete genome characterization** has allowed the creation of the **new genus *Olivavirus***
- **OLYaV** has a **high genetic diversity**, a **diagnosis challenge**
- **OLYaV untraslated regions** are most suitable for **inclusive diagnosis**
- **Psyllids** are potential **transmission vectors** of **olivaviruses**
- **OLMV** is a **new olivavirus** potentially associated to **leaf mottling**
- **Co-infections** pose a **challenge** in **etiological studies**

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Thank you!

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