

In-depth study of tomato and weed viromes reveals undiscovered plant virus diversity in an agroecosystem

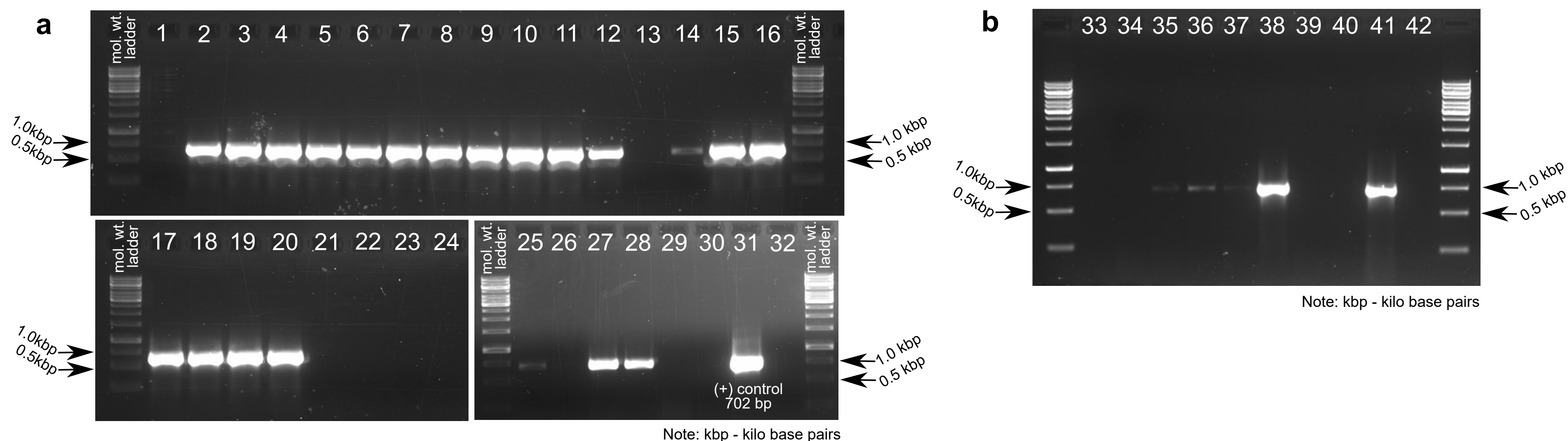
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SUPPLEMENTARY INFORMATION

Additional File 10

Supplementary Figure 5. RT-PCR, TEM and nanopore sequencing results on the confirmation of infectivity of *Plantago tobamovirus 1* (PTV1) on Solanaceae hosts.



Samples tested in the RT-PCR assay shown in **a**:

Assay controls in **a**:

- 1 - non-template control 1
- 13 - blank 1
- 21 - negative control of RNA isolation 1
- 22 - negative control of RNA isolation 2
- 23 - non-template control 1
- 24 - blank 2
- 31 - positive control (PTV1 RNA)
- 32 - negative control of RNA isolation 3

Inoculated plant	7dpi pool of inoculated leaves	14 dpi pool of inoculated leaves	14 dpi pool of young apical (systemic) leaves	21 dpi pool of young apical (systemic) leaves	28 dpi pool of young apical (systemic) leaves	35 dpi pool of young apical (systemic) leaves
<i>Solanum lycopersicum</i>	2 ^{asym}	8 ^{asym}	14 ^{asym}	25 ^{a,asym} 26 ^{b,asym}	27 ^{a,asym} 28 ^{b,asym}	29 ^{a,asym} 30 ^{b,asym}
<i>Nicotiana benthamiana</i>	3 ^{sym}	9 ^{sym}	15 ^{sym}	17 ^{sym}	19 ^{sym}	not tested
<i>Nicotiana clevelandii</i>	4 ^{sym}	10 ^{sym}	16 ^{sym}	18 ^{sym}	20 ^{sym,HTS(+)}	not tested

Note: dpi - days post inoculation, ^a10x diluted RNA, ^b100x diluted RNA, ^{asym}asymptomatic, ^{sym}symptomatic, ^{HTS(+)} was used in HTS where presence of PTV1 was confirmed. Other inoculated plants tested: 7dpi/14dpi pool of inoculated leaves: *Nicotiana tabacum* (lane 5/lane11), *Nicotiana glutinosa* (lane 6/lane12), *Chenopodium quinoa* (lane 7/-);

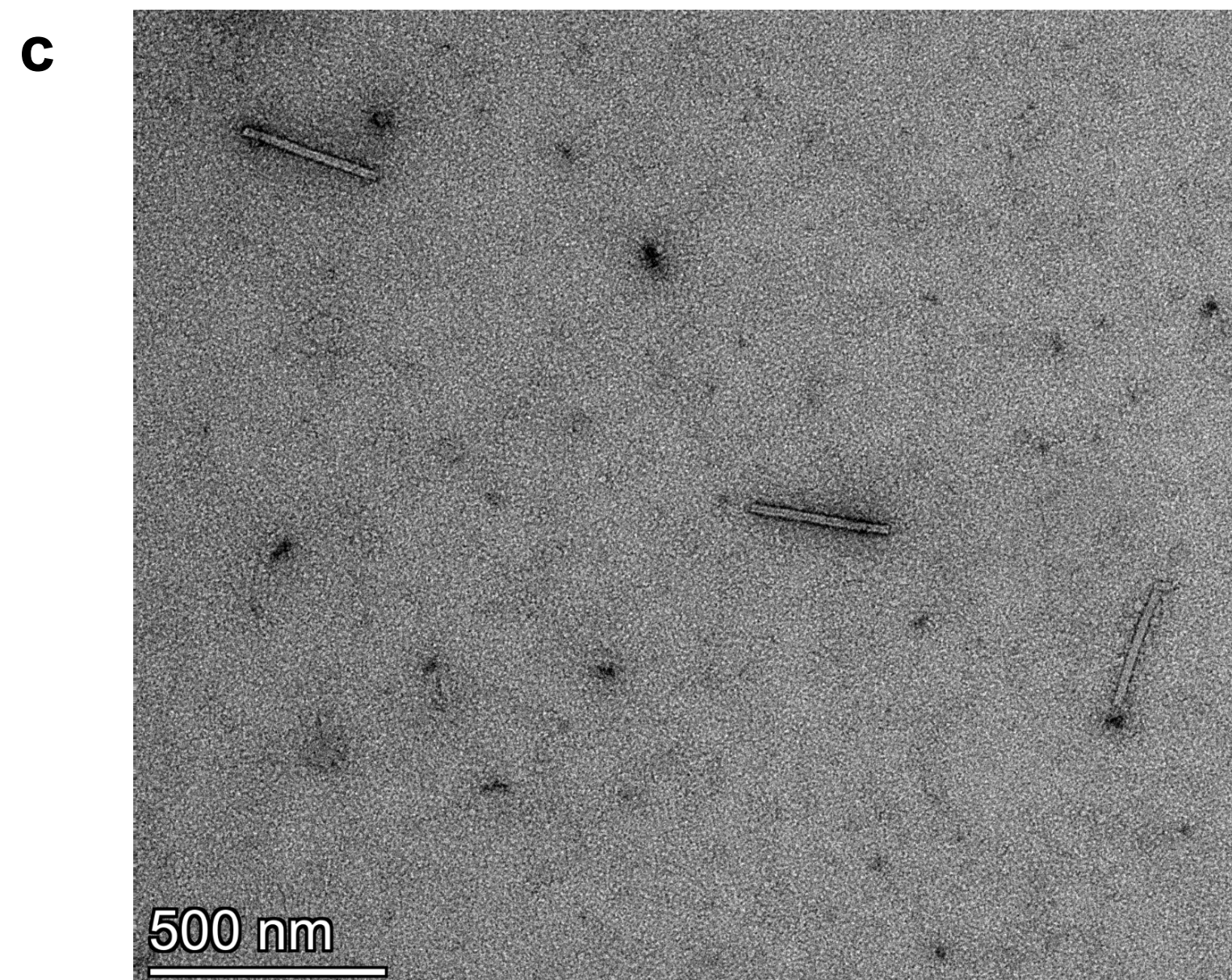
Samples tested in the RT-PCR assay shown in **b**:

Assay controls in **b**:

- 33 - non-template control
- 41 - positive control (PTV1 RNA)
- 42 - negative control of RNA isolation

Inoculated plant	28 dpi young apical (systemic) leaves from individual plants					49 dpi pool of young apical (systemic) leaves	56 dpi pool of young apical (systemic) leaves
	plant 1	plant 2	plant 3	plant 4	plant 5		
<i>Solanum lycopersicum</i>	34 ^{asym}	35 ^{asym}	36 ^{asym}	37 ^{asym}	38 ^{asym}	39 ^{asym}	40 ^{asym}

Note: RNA extracts used in this assay (**b**) were diluted 1:200, except for the positive control. dpi - days post inoculation. ^{asym}asymptomatic. ^{sym}symptomatic.



Test plant / collection dpi / individual or pooled?	No. of quality-controlled (QC) reads	No. of reads mapping to PTV1 genome	Percentage of QC reads mapping to PTV1 genome
<i>Solanum lycopersicum</i> / 28 dpi / individual plant ^a	157191	0	0.00%
<i>Solanum lycopersicum</i> / 28 dpi / individual plant ^b	160438	0	0.00%
<i>Nicotiana clevelandii</i> / 21 dpi / pooled plants ^c	159570	14216	8.91%

Note: ^alane 35 in (**b**). ^blane 37 in (**b**). ^clane 18 in (**a**).

Supplementary Figure 5. RT-PCR, TEM and nanopore sequencing results on the confirmation of infectivity of Plantago tobamovirus 1 (PTV1) on Solanaceae hosts. a, b RT-PCR assays for the detection of PTV1 in inoculated and young apical (systemic) leaves at different time points. **c** Transmission electron micrograph showing three virions of PTV1 from crude preparations of infected *Nicotiana clevelandii* tissues. **d** Number and proportion of long reads mapping to PTV1 genome, derived from selected inoculated plants.