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# Molecular and biological characterization of novel and known *Sequiviridae* members infecting lettuce

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1 **ABSTRACT**

2 High-throughput sequencing of two lettuces showing virus-like symptoms in France provided  
3 evidence of infection by members of the *Secoviridae* family. One plant (JG1) had a complex  
4 mixed infection that involved, among others, a novel *Waikavirus* (lettuce waikavirus 1) and  
5 two isolates of a sequivirus related to lettuce mottle virus (LeMoV). The second lettuce plant  
6 (JG2) was singly infected by LeMoV. Complete genomic sequences were obtained for all four  
7 isolates and, in addition, near complete genome sequences were obtained for other LeMoV or  
8 LeMoV-related isolates (from French cultivated and wild lettuces, and from a Brazilian  
9 cultivated lettuce) and for two isolates of another *Asteraceae*-infecting sequivirus, dandelion  
10 yellow mosaic virus (DaYMV). Analysis of these genomic sequences allows to propose  
11 tentative genome organization for the various viruses and to clarify their phylogenetic  
12 relationships. Sequence and host range comparisons point to significant differences between  
13 the two sequivirus isolates identified in the JG1 plant and LeMoV isolates from France and  
14 Brazil, suggesting they belong to a novel species for which the name lettuce star mosaic virus  
15 is proposed.

16 **Keywords:** Lettuce, France, Brazil, lettuce mottle virus, High-throughput sequencing,  
17 etiology.

## 18 INTRODUCTION

19 Lettuce (*Lactuca sativa* L.) is the most important annual leafy vegetable crop. It is primarily  
20 consumed fresh and is considered an important dietary vegetable worldwide. It belongs to the  
21 Asteraceae, the largest family of flowering plants. Cultivated lettuce was first documented on  
22 the walls of Egyptian tombs 4500 years ago and was soon cultivated in most parts of Europe  
23 and, later, in other parts of the world (De Vries 1997). The main producer of lettuce is Asia,  
24 especially India and China which, according to FAO in 2019, ranks first in annual production,  
25 followed by North and Central America, and Europe. In the European Union, Spain, Italy,  
26 Germany and France are the major lettuce-producing countries, with 74% of the total  
27 European lettuce production at almost 1.75 million tons in 2017 (Le Fustec 2020). In France,  
28 lettuce is grown year around in open fields, under plastic houses or glasshouses, with a total  
29 acreage of 8,300 hectares corresponding to about 500 million plants annually (Le Fustec  
30 2020).

31 A number of pests and pathogens affect lettuce crops, including viruses belonging to the  
32 genera *Alfamovirus*, *Crinivirus*, *Cucumovirus*, *Cytorhabdovirus*, *Fabavirus*, *Ophiovirus*,  
33 *Polerovirus*, *Potyvirus*, *Sequivirus*, *Tombuvirus*, *Orthospovirus* and *Varicosavirus* (Ciuffo  
34 et al. 2016; Ephytia 2019; Jadao et al. 2007; Lebeda et al. 2014; Moreno et al. 2012; Pavan et  
35 al. 2008). Many of these viruses cause epidemic diseases and considerable yield losses.  
36 Thanks to developments in high throughput sequencing (HTS) combined with specific  
37 bioinformatic tools (Lefebvre et al. 2019; Villamor et al. 2019), numerous plant viruses have  
38 been recently characterized, allowing major advances in the study of the etiology of viral  
39 diseases in perennial or annual crops (Wu et al. 2015; Maliogka et al. 2018; Maree et al.  
40 2018). However, in many cases, past the identification and genome characterization of novel  
41 viral agents, the challenge is to demonstrate their potential implication in diseases and to

42 provide information about their biology, allowing to evaluate the risks these novel agents may  
43 pose (Massart et al. 2017).

44 In recent years, several novel viruses have been characterized in lettuce crops (Ciuffo et al.  
45 2016; Svanella-Dumas et al. 2018, Verbeek et al. 2014), while the main viruses infecting  
46 lettuce in Europe or in Central America have been respectively reviewed in Moreno et al.  
47 (2012) and Pavan et al. (2008). Lettuce mosaic virus (LMV, *Potyvirus*) is probably the most  
48 important virus of lettuce worldwide (Pavan et al. 2008). LMV causes typical mosaic  
49 symptoms that may occasionally turn necrotic. However, some other lettuce viruses can cause  
50 almost indistinguishable mosaic symptoms, such as lettuce mottle virus (LeMoV) and  
51 dandelion yellow mosaic virus (DaYMV), both of them from the genus *Sequivirus* in the  
52 family *Secoviridae* (Jadao et al. 2007). DaYMV was the first sequivirus reported in lettuce  
53 and in plants of dandelion (*Taraxacum officinale*) in different European countries (Kassanis  
54 1944, 1947; Bos et al. 1983; Jadao et al. 2007). LeMoV, differing from DaYMV in host  
55 range, is a distinct sequivirus infecting lettuce in Brazil and in Chile (Krause-Sakate et al.  
56 2005; Jadao et al. 2007). In Brazil, and in particular in Sao Paulo State, LeMoV was in the  
57 early 2000s the most frequent virus in lettuce crops (De Marchi et al. 2012, Krause-Sakate et  
58 al. 2007) but it has so far never been reported outside of South America. For both DaYMV  
59 and LeMoV, only very short, partial genome sequences are available (Jadao et al. 2007) and  
60 there is very little information available on their genetic variability.

61 We report here the biological and molecular characterization by HTS of several secoviruses  
62 from wild and cultivated lettuce in France and Brazil and from dandelion in Slovakia. Besides  
63 providing the first complete genomic sequence for LeMoV and near-complete genome  
64 sequences for DaYMV, the results presented here describe a novel *Sequivirus* and a novel  
65 *Waikavirus* detected in French cultivated lettuces.

66

67

**MATERIALS AND METHODS****68 Plant samples and virus isolates**

69 To identify the etiological agent(s) involved, three cultivated lettuces (*Lactuca sativa* L.)  
70 presenting systemic mosaic symptoms with some necrotic points were analyzed by HTS. The  
71 first one (cv. Shangore, JG1) was collected in 2014 near Sainte-Livrade (southwestern  
72 France) and the second one (cv. Guétary, JG2) came from an organic plot under a plastic  
73 greenhouse and was collected in 2015 near Angers (western France). The last (21-015C,  
74 variety unknown) was collected in southern France in 2022. The mosaic symptoms on the  
75 JG2 plant, shown in Fig 1A, had more pronounced necrosis than those on the JG1 plant, while  
76 besides mosaic, the 21-015C plant showed stunting and leaf deformation.

77 Several isolates of lettuce mottle virus (LeMoV) were analyzed. The P22 isolate was obtained  
78 from an asymptomatic wild lettuce plant (*L. serriola*) collected in spring 2010 as part of a  
79 plant virus metagenomic survey in Villenave d'Ornon (southwestern France) (Svanella-  
80 Dumas et al. 2018). The second isolate (SP) was from a cultivated lettuce collected in 2013 in  
81 Piedade County, Sao Paulo State, Brazil. This isolate was propagated in *Chenopodium quinoa*  
82 or lettuce (cv. Trocadéro) plants by sap inoculation before HTS analysis. The third LeMoV  
83 isolate, LIB3, was from a wild lettuce (*Lactuca saligna*) showing leaf yellowing and  
84 discolorations collected in 2020 in Villenave d'Ornon.

85 Two isolates of dandelion yellow mosaic virus (DaYMV) in mixed infection, RNA24P-1 and  
86 RNA24P-2, were identified by HTS from a dandelion plant collected in 2018 at the border of  
87 a cultivated field near Bratislava, Slovakia.

**88 Host range study and sap inoculation of viruses**

89 In order to characterize the novel viruses identified, leaf pieces from the original JG1 plant  
90 were ground 1:3 (wt/vol) in 0.05M potassium phosphate buffer pH 8.0. Activated charcoal

91 and carborundum (400 mesh) were added (100mg/ml homogenate) and the mixture was used  
92 for sap-inoculation of plants of *L. sativa* (cv. Trocadero) or of ten plant species belonging to  
93 the families *Asteraceae*, *Solanaceae*, *Chenopodiaceae* and *Amaranthaceae*. The LeMoV  
94 isolate identified in the JG2 plant was sap-transmitted to lettuce (cv. Trocadéro), *Nicotiana*  
95 *benthamiana* and *Gomphrena globosa* plants using the buffer described in Jadao et al. (2007).  
96 The host range study was repeated at least twice for each viral isolate. All plants were kept  
97 under greenhouse conditions and monitored for symptoms development up to one-month  
98 post-inoculation.

99 The presence of the viruses in upper non-inoculated leaves of the test plants was evaluated  
100 using specific RT-PCR assays for each virus using total nucleic acids (TNA) extracted from  
101 fresh leaves according to protocol 1 of Foissac et al. (2005). The sequences of the various  
102 amplification primers used [New waika-F / New waika-R (for the new waikavirus) and  
103 NSeco-varA-F / NSeco-varA-R or NSeco-varB-F / NSeco-varB-R (that discriminate between  
104 the two variants of the new sequivirus)] are provided in Supplementary Table S1. These  
105 primer pairs target fragments of respectively 385, 445 or 546 nucleotides (nt) in the viral  
106 RNA-dependent RNA polymerase (RdRp) gene of each virus. The presence of LeMoV was  
107 similarly checked using primer pair LeMoV3 (Jadao et al. 2007) and LeMoV4-multi  
108 (Supplementary Table S1) which is adapted from Jadao et al. (2007) so as to improve  
109 inclusiveness and to allow amplification of isolates JG2, P22, SP described here and of  
110 previously analyzed Brazilian LeMoV isolates (DQ675190-91). This primer pair amplifies a  
111 667 nt fragment of the RdRp gene.

112 For each RT-PCR assay, cDNA was first synthesized from 5µl of TNA using dT18 and pdN6  
113 primers and the Revertaid Reverse Transcriptase (200U/µl) according to the manufacturer  
114 recommendations (Thermo Fisher Scientific, Illkirch, France). In a second step, the PCR was  
115 carried out using 3 µl of cDNA in a 25-µl reaction volume using 1 U of DyNAzymeII™ DNA

116 polymerase in accordance with the manufacturer recommendations (Thermo Fisher  
117 Scientific). The annealing temperature and duration of the 72°C extension step are provided in  
118 Supplementary Table S1. PCR products were analyzed by non-denaturing 1% agarose gel  
119 electrophoresis with ethidium bromide staining and, if needed, directly sequenced (Eurofins  
120 Genomics, Ebersberg bei München, Germany) to verify the specificity of the amplification  
121 reaction.

### 122 **HTS analysis, genome assembly and completion of the genome of the various viral** 123 **isolates**

124 Double-stranded RNAs (dsRNAs) were purified from fresh symptomatic leaves of the  
125 original JG1, JG2, P22 and LIB3 plants as described (Marais et al. 2018). Purified dsRNAs  
126 were submitted to a random amplification (Marais et al. 2018) and sequenced on Illumina  
127 platforms (2×250 or 2x150 nt). Total RNAs were purified from *N. benthamiana* leaves  
128 infected by the LeMoV-JG2 isolate using the protocol of Chang et al. (1983) and sequenced  
129 without ribodepletion on an Illumina HiSeq2000 (2×150 nt). Additionally, total RNAs were  
130 extracted from fully developed dandelion leaves as mentioned in Tomasechova et al. (2020)  
131 and sequenced on an Illumina MiSeq (2×250 nt). Total RNAs extracted from the 21-015C  
132 lettuce were similarly sequenced on the Illumina iSeq100 and MiSeq platforms (2x150nt).

133 Following demultiplexing and quality trimming, HTS reads from the various samples were  
134 analyzed using CLC Genomics Workbench 8 and later versions  
135 (<https://www.qiagenbioinformatics.com>) and the VirAnnot pipeline (Lefebvre et al. 2019). *De*  
136 *novo* assembled contigs were annotated by BlastN and BlastX analyses against the GenBank  
137 database using a 10<sup>-3</sup> *e*-value cut-off. Viral contigs were further extended by several rounds of  
138 mapping of residual reads and by integration of smaller contigs to yield scaffold covering  
139 most of the viral genomes.



140 The 5' end of viral genomes was determined using a 5' RACE kit (Takara Bio  
141 Europe/Clontech, Saint-Germain-en-Laye, France) and specific reverse primers designed on  
142 the reconstructed scaffold of each virus (Supplementary Table S1). To complete the 3' end of  
143 the genomes of the sequiviruses, that do not contain a poly-A tail, TNA were extracted and  
144 polyadenylated (Ambion/ThermoFisher Scientific, Illkirch, France). The polyadenylated  
145 RNAs were then submitted to a reverse transcription using LD-polyT as a reverse primer and  
146 the genome 3' end was then amplified by PCR using a forward-specific internal primer  
147 designed from the genome scaffold sequenced contig (Supplementary Table S1) and the LD-  
148 prime primer, following the protocol described by Youssef et al. (2011). The same procedure  
149 was used to determine the *Waikavirus* 3' genome end, only omitting the polyadenylation step  
150 since waikavirus genomes are polyadenylated.

151 Internal gaps and regions of low coverage were determined or confirmed by direct sequencing  
152 of RT-PCR fragments obtained using internal primers designed from the sequences of  
153 contigs. For LeMoV-JG2, the only gap identified was in the 3' part of the genome, in a C-rich  
154 region with low coverage. Two combinations of primers (LeMoV-3Race1 / LeMoV-gap-R1  
155 and LeMoV-3Race1 / LeMoV-gap-R2) (Supplementary Table S1), amplifying PCR products  
156 of respectively 199 and 573 nt were used to confirm the genome sequence in this short region.  
157 The nearly genomic sequence of the LeMoV isolate P22 was completed after designing six  
158 pairs of primers (Supplementary Table S1) allowing to close six small internal gaps in the  
159 HTS scaffold.

160 All amplified fragments were visualized on non-denaturing agarose gels and directly  
161 submitted to Sanger sequencing on both strands (Eurofins Genomics) or, when needed,  
162 sequenced after cloning in the pGEM-T Easy vector (Promega, Charbonnières-Les Bains,  
163 France).

164 The nearly complete genome of the LeMoV isolate SP was obtained by RT-PCR-based  
165 genome walking, using total RNAs extracted from infected *C. quinoa* leaves with the Total  
166 RNA Purification Kit (Norgen Biotek, Thorold, Canada). The primers used (Supplementary  
167 Table S1) were designed based on the genome sequence of the LeMoV P22 isolate. The one-  
168 step RT-PCR reactions were performed using 2.5 µl of RNA, 1 µM of each primer and 0.03  
169 units of Avian myeloblastosis virus reverse transcriptase (AMV RT, Promega, Charbonnières-  
170 les-Bains, France) in a final volume of 25 µl of 1x PCR master mix (Fermentas, Vilnius,  
171 Lithuania). The annealing temperature for each primer pair is provided in Supplementary  
172 Table S1. PCR products were analyzed as described above, purified from agarose gels using  
173 the QIAquick Gel Extraction Kit (Qiagen, Courtaboeuf, France) and sequenced using an ABI  
174 3730 DNA Analyser (Applied Biosystems, Illkirch, France) using a BigDye Terminator v3.1  
175 Cycle Sequencing kit (Applied Biosystems).

## 176 **Sequence comparisons and phylogenetic analyses**

177 Multiple nucleotide (nt) or deduced amino acid (aa) sequence alignments were performed  
178 using ClustalW (Thompson et al. 1994) implemented in MEGA 7 (Kumar et al. 2016). Strict  
179 identity nt or aa distances were calculated from these alignments and neighbor-joining  
180 phylogenetic trees were reconstructed using Mega 7 with bootstrap analysis (500 replicates)  
181 to evaluate branch validity.

## 182 **RESULTS**

### 183 **Viruses detected by HTS in the French JG1 and JG2 lettuces**

184 During the spring of 2014 and 2015, two symptomatic lettuces (JG1, cv. Shangore and JG2  
185 cv. Guétary) showing systemic mosaic symptoms were observed during surveys. Both  
186 varieties are resistant to lettuce downy mildew (*Bremia lactucae* Regel) but only JG2 is

187 resistant to lettuce mosaic virus (LMV) and to the lettuce aphid, *Nasonovia ribisnigri*. The  
188 symptoms on the JG2 lettuce included some necrotic points (Fig. 1A) that were much less  
189 present on the JG1 plant. Symptomatic leaves of both plants were used to mechanically  
190 inoculate lettuce plants (cv. Trocadéro), which developed systemic mosaic symptoms,  
191 confirming suspicions of viral infection. In order to identify the virus(es) involved, dsRNAs  
192 were purified from 0.75g of fresh leaves from the original JG1 and JG2 plants and analyzed  
193 by HTS. Following demultiplexing and quality trimming, the reads were assembled into  
194 contigs that were annotated by BlastN and BlastX analyses, providing evidence for a mixed  
195 infection by several agents for the JG1 lettuce and for a single infection in JG2 (Table 1).

196 For JG1, the contig integrating the most reads (26,980) and showing the highest average  
197 coverage (449x), showed the highest BlastN identity (49% nt identity) with bellflower vein  
198 chlorosis virus (BVCV, Seo et al. 2015), a recently described member of the genus  
199 *Waikavirus*. A second contig, integrating 12,327 reads for a 283x average coverage, showed  
200 the closest homology (54% nt identity) with carrot chordovirus 1 (Adams et al. 2014). The  
201 complete genome of this viral isolate has since been completed, allowing the description of a  
202 new viral species, lettuce chordovirus 1 (LeCV1, Svanella-Dumas et al. 2018) in the family  
203 *Betaflexiviridae*. The last two contigs integrating respectively 4,198 and 5,227 reads (average  
204 coverage of respectively 92x and 102x) showed highest BlastN nt identities of 54% with  
205 parsnip yellow fleck virus (PYFV, Turnbull-Ross et al. 1993) and 47% with carrot necrotic  
206 dieback virus (CNDV, Menzel et al. 2008), two members of the *Sequivirus* genus,  
207 respectively. Following the extension of these two contigs by several rounds of reads mapping  
208 and aggregation of additional smaller contigs, two scaffolds of respectively 8,961 nt and  
209 9,158 nt, were reconstructed, showing 79.8% nt identity. Taken together these results showed  
210 that the original JG1 lettuce had a mixed infection involving LeCV1, a tentatively novel  
211 *Waikavirus* and two related and tentatively novel sequiviruses.

212 For JG2, the largest viral contig identified from dsRNA-derived reads, was 1,996-nt long (96x  
213 average coverage, 891 reads) and showed 50% nt identity with CNDV (data not shown).  
214 Given the very incomplete assembly of the viral genome, a second HTS run was performed  
215 on this viral isolate using total RNAs purified from infected *N. benthamiana* leaves. Using  
216 this second dataset, evidence for a single viral infection was obtained. A single long viral  
217 contig was obtained and further extended by rounds of reads mapping into an 8,927-nt long  
218 scaffold integrating 119,029 viral reads (1,790x average coverage) and sharing 50% nt  
219 identity with CNDV (Table 1). Interestingly, this scaffold shared 85.4 and 86.3% nt identity,  
220 respectively (98.4 and 97.9% aa identify, respectively) in a 562 nt region shared with the two  
221 partial LeMoV sequences available (DQ675190 and DQ675191), suggesting that the viral  
222 isolate identified in the JG2 plant might represent the first identification of LeMoV in  
223 cultivated lettuce outside of south America.

#### 224 **Genome organization of the *Waikavirus* identified in the JG1 lettuce**

225 Following further rounds of reads assembly and aggregation of shorter contigs, a nearly  
226 complete genome scaffold of 11,852 nt was assembled for the tentative *Waikavirus* identified  
227 in the JG1 plant. The missing terminal genome ends were determined by 5'-RACE and 3'  
228 LD-polyT / LD-prime PCR (Youssef et al. 2011) using the purified dsRNAs from the original  
229 plant as a template and specific primers designed from the scaffold sequence (Supplementary  
230 Table S1). The completed genome is 11,929 nt in length, excluding the 3'poly (A) tail (Fig.  
231 2A) and has been deposited in GenBank under the MT348710 accession number. The genome  
232 organization is similar to that of other waikaviruses, with a single large open reading frame  
233 (ORF) of 10,389 nt encoding a polyprotein of 3,463 aa (388.5 kDa). The 5' and 3'  
234 untranslated regions (UTRs) are respectively 463 nt and 1,078 nt long. The presence of  
235 additional potential ORFs has been reported for some waikaviruses (Shen et al. 1993; Firth et  
236 al. 2008). The predicted short overlapping ORFX was identified starting at genome position

237 464, encoding an 88 aa protein sharing 73.3% identity with the corresponding protein of  
238 BVCV and variable conservation levels with other genus members (Supplementary Figure 1).  
239 On the other hand, the long 3' UTR of the JG1 waikavirus does not contain the small ORF2  
240 and ORF3 described in rice tungro spherical virus (RTSV) by Thole et al. (1996). In  
241 waikaviruses and sequiviruses, the single large polyprotein encodes functional proteins  
242 released by post-translational cleavages mediated by the viral protease (Pro). From the N-  
243 terminal polyprotein end, these are a P1 protein of unknown function, the three coat protein  
244 subunits (CP1, CP2, and CP3), an NTP-binding helicase (NTB), the 3C-like protease and an  
245 RdRp. The polyprotein cleavage sites have been experimentally identified for maize chlorotic  
246 dwarf virus (MCDV) and RTSV (Reddick et al. 1997; Shen et al. 1993). They are relatively  
247 conserved between genus members and generally involve a glutamine (Q) residue at the -1  
248 position (Sekiguchi et al. 2005; Sanfaçon et al. 2011). Considering the homologies between  
249 the lettuce virus polyprotein and those of other waikaviruses, it was possible to propose  
250 tentative cleavage sites for its polyprotein using a ClustalW-generated multiple alignment of  
251 the various polyproteins (Fig. 2 and Table 2). A comparison of the amino acid sequence at  
252 each of the proteolytic cleavage sites of the lettuce virus (hereafter referred to as LWV1-JG1)  
253 showed that glutamine was conserved at the -1 position, with different amino acids in the +1  
254 position (Q/S, Q/T and Q/D) depending on the cleavage site. Comparisons between the  
255 LWV1-JG1 polyprotein sequence and those of other waikaviruses confirmed the presence of  
256 the expected conserved domains in the Hel-Pro-Pol module that is commonly used to  
257 characterize picorna-like viruses (Le Gall et al. 2008). This includes the A "GAPGVGKS" (aa  
258 1806 to 1813), B "DD" (aa 1857-1858) and C "KGKHCTSKYVFSCTN" (aa 1893 to 1907)  
259 helicase motifs (Le Gall et al. 2008), the 3C-like protease catalytic triad of histidine, aspartate  
260 and cysteine (Reddick et al. 1997) present at amino acids 2670, 2707 and 2801 and the A  
261 "DYAKFDGIGSP" (aa 3149 to 3159), B "SGFSMTVIFNS" (aa 3210 to 3220) and C

262 “YGDD” (aa 3258 to 3261) motifs (Reddick et al. 1997; Le Gall et al. 2008) characterizing  
263 the RdRp domain.

#### 264 **Genome organization of the sequivirus isolates identified in the JG1 lettuce**

265 As indicated above, two long scaffolds representing sequivirus isolates could be reconstructed  
266 for the JG1 plants, named here JG1-A (8,961 nt) and JG1-B (9,158 nt). The whole genome of  
267 the JG1-B was completed by 5'-RACE and 3' LD-polyT / LD-prime PCR as described above  
268 for the *Waikavirus* sequence, with the only exception that the genome 3' end was determined  
269 after polyadenylation since sequiviruses do not possess a 3' polyA. No specific efforts were  
270 made to complete the JG1-A genome sequence. The JG1-B complete genome is 10,183 nt  
271 long and has been deposited in GenBank, together with the JG1-A contig sequence  
272 (MT348706 and MT348705, respectively). As expected, the JG1-B genome harbors a single  
273 large ORF of 9,054 nt encoding a predicted polyprotein of 3,018 aa (337.5 kDa), bordered by  
274 5' and 3' UTRs of 150 nt and 980 nt, respectively (Fig. 2B). Conserved protein motifs were  
275 identified at the expected locations in the JG1-B polyprotein. This includes the A  
276 “GSPGVGKS” (aa 1430 to 1437), B “DD” (aa 1481 to 1482) and C “KGRTFSSKYIFSTTN”  
277 (aa 1517 to 1531) helicase motifs, or the B “SGFPMTVIFNS” (aa 2555 to 2565) and C  
278 “YGDD” (aa 2603 to 2606) RdRp motifs. However, polyprotein cleavage sites are much less  
279 conserved between members of the *Sequivirus* genus (Sanfaçon et al. 2011), so that it was not  
280 possible to propose candidates for all tentative cleavage sites of the JG1-B polyprotein (Fig.  
281 2B and Table 2) from a comparison with those reported for PYFV (Turnbull-Ross et al.  
282 1993). Nevertheless, four candidate sites liberating the P1 protein and the three CP subunits  
283 could be proposed (N/P, Q/G, Q/G and Q/A) at aa position 383/384, 575/576, 799/800 and  
284 1035/1036 of the polyprotein, respectively (Fig. 2B and Table 2). With its 79.8% overall nt  
285 identity with the JG1-B sequence, the partial JG1-A isolate sequence revealed complete  
286 conservation of the genome features outlined above.

**287 Genome organization of the LeMoV isolate identified in the JG2 lettuce**

288 The genome ends of the 8,927 nt contig for the LeMoV isolate assembled from the sequence  
289 data generated for the JG2 lettuce were determined by 5'-RACE and 3' LD-polyT / LD-prime  
290 PCR. As for the JG1-B sequivirus, it was necessary to add a poly-A tail at the 3' genome end.  
291 The completed JG2 LeMoV genome has been deposited in GenBank (MT348707) and is  
292 9,927 nt long and has 5' and 3' UTRs of respectively 158 and 704 nt (Fig. 2C). It harbors a  
293 single large ORF of 9,066 nt encoding a predicted polyprotein of 3,022 aa (336.1 kDa), which  
294 has all the expected conserved protein motifs at the expected positions. Indeed, all aa  
295 sequences of motifs are similar to those identified for both isolates JG1-A and JG1-B except  
296 for the motif C of the helicase region for which a serine replaces an arginine at the 6<sup>th</sup>  
297 position. As for the JG1-B sequence, it was only possible to predict the four tentative  
298 cleavage sites liberating the P1 protein and the three CP subunits (Fig. 2C and Table 2). Two  
299 out of four predicted cleavage sites Q/G (577/578, between CP1 and CP2) and Q/A  
300 (1037/103, between CP3 and Helicase) are identical to those predicted for JG1-B.

**301 Genetic diversity of JG1-A/JG1-B-like isolates and of lettuce mottle virus (LeMoV)**

302 Two nearly complete *Sequivirus* genome sequences related to the JG1-A/JG1-B isolates were  
303 assembled from the reads obtained from a symptomatic cultivated lettuce (21-015C, cv.  
304 unknown) collected in southern France in 2022. These two contigs are respectively 10107 nt  
305 (21-015C-A, OQ446446) and 10066 nt (21-015C-B, OQ446447) and had an average  
306 coverage of respectively 460 and 343x (Table 1). No specific effort was made to complete the  
307 5' and 3' genome UTRs. The two sequences show 23.7% nt divergence with each other and  
308 respectively 20.8% and 25% divergence with JG1-A. On the other hand, 21-015C-A shows  
309 only 8.6% divergence with JG1-B, while the corresponding value for 21-015C-B is 23.9%.  
310 Genomic organization and key protein motifs are conserved between these two new isolates

311 and the JG1 isolates, with only one polymorphism observed at the P1/CP1 tentative cleavage  
312 site (Table 2)

313 Nearly complete genome sequences were obtained for five other LeMoV isolates, P22  
314 assembled from the reads obtained from an asymptomatic wild lettuce plant (*L. serriola*)  
315 collected in spring 2010 as part of a plant virus metagenomic survey in Villenave d'Ornon  
316 (southwestern France) (Svanella-Dumas et al. 2018), SP assembled from the reads from a  
317 cultivated lettuce collected in 2013 in Sao Paulo State, Brazil, 21-015C assembled from the  
318 reads of the cultivated lettuce mentioned above and LIB3-A and LIB3-B assembled from the  
319 reads from a wild *Lactuca saligna* collected in southwest France in 2020. The P22 contig of  
320 9,012 nt (GenBank MT348708) integrates 2,517 reads (78x average coverage), while a large  
321 part of the SP isolate genome (8,366 nt) was determined by primer walking along the genome  
322 (GenBank MT348709). The LIB3-A (OQ446443) and LIB3-B (OQ446444) sequences are  
323 9150 nt long while the 21-015C sequence (OQ446445) is 9807 nt long but contains a short 24  
324 nt internal gap at the position of the C rich region in which a similar gap was closed by PCR  
325 for the JG2 sequence. No specific efforts were made to determine the missing terminal  
326 sequences for any of these five LeMoV sequences. The JG2 genomic sequence shows  
327 between 11.1% and 14.4% nt divergence (2.0-3.5% aa divergence) with these 5 novel near  
328 complete LeMoV sequences, which in turn show between 11.4% and 15% nucleotide  
329 divergence (2.2-3.9% aa divergence) with each other. All conserved protein motifs and  
330 predicted cleavage sites are identical for the various LeMoV isolates (Table 2).

### 331 **Genomic organization of a DaYMV isolate identified in a Slovak dandelion**

332 As described above, two viral contigs with homologies to sequiviruses could be assembled  
333 from the reads obtained from the dandelion RNA24P plant from Slovakia. These contigs  
334 respectively integrate 3,824 and 1,003 reads, corresponding to average coverages of 60-fold  
335 and 15-fold, respectively and showed the highest homology (49% nt identity) with CNDV



336 (Table 2). Further assembly efforts yielded two large scaffolds named RNA24P-1 and  
337 RNA24P-2 of 9,786 and 9,795 nt long, respectively, and sharing 77% and 81.7 % nt sequence  
338 identity (95% and 96.2 % aa), respectively, with the only available DaYMV partial sequence  
339 (DQ675189, 788 nt long). No specific efforts were made to determine the 5' and 3' terminal  
340 regions of the nearly complete RNA24P-1 and RNA24P-2 genomes and both large contigs  
341 have been deposited in GenBank (MT559283 and MT559284, respectively). These results  
342 constitute the first identification of DaYMV isolates in dandelion in Slovakia. The RNA24P-1  
343 genome organization is similar to that of other sequiviruses, with a single large ORF of 9,102  
344 nt encoding a polyprotein of 3,035 aa (338.7 kDa). The incomplete 5' and 3' UTRs are  
345 respectively 149 nt and 533 nt long (Fig. 2D), indicating that the amount of missing sequence  
346 information is likely limited. The RNA24P-2 genome has an ORF of 9,105 nt encoding a  
347 polyprotein which is one aa longer than that of RNA24P-1 in the 3C-like protease domain. All  
348 polyprotein conserved amino acid motifs are identical to those identified for LeMoV isolates,  
349 except for the helicase A motif in which a lysine replaces a serine at position 2. As for LSMV  
350 and LeMoV, it was only possible to propose candidates for the first four polyprotein cleavage  
351 sites of DaYMV (K/P, Q/G, Q/M/S and Q/G at aa positions 388/389, 580/581, 804/805 and  
352 1041/1042, respectively; Fig. 2D and Table 2), with an M/S polymorphism between the two  
353 isolates at the +1 position of the CP2-CP3 cleavage site.

#### 354 **Sequence comparisons and phylogenetic relationships of the various viruses identified in** 355 **the analyzed wild and cultivated lettuces**

356 To clarify the taxonomic position of these various viruses identified above, sequence  
357 comparisons and phylogenetic analyses were performed either with the taxonomically  
358 relevant Pro-Pol region (defined as spanning the part of the polyprotein between the  
359 conserved CG protease and GDD polymerase motifs) or with the coat protein subunits for all  
360 viruses in the genera *Waikavirus* and *Sequivirus*, as well as for representative members of the

361 genera *Cheravirus*, *Comovirus*, *Cholivirus*, *Fabavirus*, *Nepovirus*, *Satsumavirus*,  
362 *Stralaravirus*, *Torradovirus* and for unassigned viruses in the *Secoviridae* family (Sanfaçon et  
363 al. 2020) (Fig. 3A and 3B). The trees unambiguously placed the JG1 *Waikavirus* with other  
364 genus members with strong bootstrap support and the JG1-A/JG1-B-like and LeMoV  
365 sequences within the *Sequivirus* genus, again with strong bootstrap support (Fig. 3A and 3B),  
366 confirming the assignation of these various viruses to this genus. In the case of the  
367 sequiviruses, only very short partial genome sequences were previously available for LeMoV  
368 and DaYMV (Jadao et al. 2007). Integration of these partial sequences in the phylogenetic  
369 trees confirm the identification of the JG2 isolate as LeMoV, together with the P22, SP, LIB3-  
370 A, LIB3-B and 21-015C isolates, with a tight, bootstrap-supported clustering (Fig. 3A) and  
371 shows that DaYMV unambiguously clusters away from the bootstrap-supported cluster  
372 formed by the the JG1-A, JG1-B, 21-015C-A and 21-015C-B isolates, ruling out their  
373 interpretation as DaYMV isolates (Fig. 3A).

374 The aa sequence identity levels between the JG1 *Waikavirus* and other waikaviruses are  
375 below 64% and 56% for the Pro-Pol region and for the CPs, respectively (Supplementary  
376 Table S2). Considering the species molecular discrimination criteria in the *Secoviridae* family  
377 (less than 80% aa sequence identity in the Pro-Pol region and less than 75% identity in the  
378 CPs) (Sanfaçon et al. 2009; Thompson et al. 2017), the isolate identified in the JG1 lettuce  
379 should be considered as belonging to a novel species, for which the name lettuce waikavirus 1  
380 (LWV1) is proposed.

381 Concerning the JG1-A/B and 21-015C-A/B isolates, they share with each other 86.2-97.5%  
382 and 92.1-99.7% aa sequence identity in their Pro-Pol and CPs, respectively. They should thus  
383 be considered as isolates belonging to the same virus species. As compared with other known  
384 sequiviruses, they share at most 76.9% identity in the Pro-Pol region (with LeMoV isolates),  
385 which is below the 80% species demarcation criterion (Sanfaçon et al. 2009; Thompson et al.

386 2017) (Supplementary Table S3). However, when comparing the CPs, an aa identity value of  
387 82.2-84.2% is observed with LeMoV isolates, higher than the 76% species cut-off value. This  
388 suggests that other species discrimination criteria need to be taken into consideration  
389 (Thompson et al. 2017), in order to be able to decide on their taxonomic situation. We  
390 therefore decided to compare the experimental host range of LeMoV with that of the JG1-A  
391 and JG1-B isolates.

### 392 **Comparative host range analysis for various sequiviral isolates**

393 Despite repeated attempts using various buffers and inoculation conditions, all efforts to  
394 transmit LWV1 from the JG1 plant to lettuce (cv. Trocadéro) failed (data not shown). The  
395 same was observed for LeCV1 (Svanella-Dumas et al. 2018). These observations are  
396 consistent with the notion that waikaviruses are not mechanically sap-transmissible because  
397 they are confined to phloem tissue (Reavy et al. 1993). On the other hand, variants JG1-A and  
398 JG1-B were readily transmitted from the JG1 plant and induced systemic mosaic and star  
399 chlorosis symptoms on inoculated lettuce (cv. Trocadéro) (Figure 1F). These initial  
400 transmission efforts also allowed to separate the JG1-A and JG1-B isolates as demonstrated  
401 using isolate-specific RT-PCR assays (Supplementary Table 1). The JG1-A and JG1-B  
402 isolates, as well as the JG2 LeMoV isolate, were sap-inoculated to plants belonging to ten  
403 species in four families, some of which are reported as differential hosts between LeMoV and  
404 DaYMV (Jadao et al. 1997, Bos et al. 1983; Table 3). The expression of symptoms was  
405 monitored over a one-month period and the presence of the various viruses was verified in  
406 non-inoculated upper parts of test plants by specific RT-PCR assays. The results of these  
407 experiments are presented in Table 3.

408 In summary, the JG1-A and JG1-B variants were found to accumulate in six and seven of the  
409 ten tested species, respectively. For both isolates, systemic mosaic symptoms were observed  
410 on lettuce (cv. Trocadero), endive (*Chicorium endivia* cv. Anjou) (Fig. 1E) and common

411 sowthistle (*Sonchus olearaceus*), while asymptomatic accumulation was observed in *N.*  
412 *benthamiana*, *N. clevelandii* and *Gomphrena globosa* (very occasionally only for JG1-A).  
413 JG1-B, but not JG1-A, also accumulated in *C. quinoa* in which it induced yellow systemic  
414 spots (Figure 1D).

415 In parallel experiments, inoculation of the JG2 LeMoV isolate yielded results only partially  
416 comparable to those reported by Jadao et al. (1997) for the Brazilian AF197 isolate since  
417 contrary to AF197, JG2 caused systemic mosaic symptoms in *C. endivia* and only caused  
418 symptomless infection in *G. globosa* (Table 1). Another noteworthy difference is that contrary  
419 to AF197, which systemically infected and caused local lesions and systemic symptoms in *C.*  
420 *quinoa*, JG2 almost never infected this host. However, a *C. quinoa* plant was identified with  
421 JG2 infection in independent experiments than those reported in Table 3. The virus from this  
422 plant could then readily be transmitted to *C. quinoa* but with only a drastically reduced  
423 efficiency to lettuce (not shown).

424 Taken together these results show that similar to the situation with LeMoV or with DaYMV,  
425 there is significant inter-isolate variability in the host range of the JG1-A and JG1-B isolates.  
426 The only host that consistently separated the JG1-A and JG1-B isolates from LeMoV isolates  
427 was *N. clevelandii*, which was symptomlessly infected by the former, but not by the latter.  
428 Differences also exist with DaYMV, for which multiple isolates are reported to cause local  
429 lesions in *C. amaranticolor* and to be unable to infect *S. oleraceus* (Table 3, Bos et al. 1983).

430

## DISCUSSION

431 In the current study, the use of HTS data in combination with 3' and 5' RACE and  
432 confirmation by RT-PCR assays for regions of low coverage allowed us to assemble complete  
433 genomic sequences for three *Secoviridae* family members from symptomatic lettuces  
434 cultivated in France. Similar approaches were also used to obtain near-complete genome

435 sequences for additional isolates of the same viruses from wild or cultivated lettuces in France  
436 or in Brazil as well as the first near-complete genomes for yet another *Secoviridae* member,  
437 DaYMV from a dandelion from Slovakia.

438 For the *Waikavirus* found in the cultivated JG1 lettuce, its genome characterization and  
439 phylogenetic analysis show that it belongs to a new species, for which the name lettuce  
440 waikavirus 1 is proposed. The unsuccessful results at mechanical transmission suggest that  
441 like RTSV, this new virus could be confined to phloem tissues and not mechanically  
442 transmissible (Reavy et al. 1993). The biology and epidemiology of waikaviruses remain  
443 poorly understood, likely in part due to their phloem restriction and reliance on specific  
444 arthropod vectors for efficient transmission (Reddick et al. 1997). Some waikaviruses also  
445 serve as helper viruses for sequiviruses or caulimoviruses (Elnagar et al. 1976, Thompson et  
446 al. 2017). However, some other sequiviruses such as DaYMV and LeMoV are reported to be  
447 transmitted by aphids in the absence of a helper virus (Jadao et al. 2007). In the case of the  
448 JG1 plant, given the mixed infection involving both LWV1 and the JG1-A and JG1-B  
449 sequivirus isolates, it is not possible to know whether transmission complementation effects  
450 exist or not between these various *Secoviridae* members. However, the identification of the  
451 21-015C-A and -B isolates in a wild lettuce and in the absence of a *Waikavirus* suggests that  
452 similar to LeMoV these isolates do not require the assistance of a *Waikavirus* for  
453 transmission.

454 Considering the 80% cut-off molecular criterion in the Pro-Pol region defining species within  
455 the family *Secoviridae* (Sanfaçon et al. 2009; Thompson et al. 2017), the JG1-A, JG1-B, 21-  
456 015C-A and 21-015C-B isolates should all be considered as belonging to a single new species  
457 in the genus *Sequivirus*. However, when considering the 76% cut-off value that applies to the  
458 CPs, they should be considered as isolates of LeMoV. They are however unambiguously  
459 different from DaYMV, the only sequivirus known to date from lettuce in Europe. At the

460 same time, the JG2, P22, LIB3-A, LIB3-B and 21-015C isolates from cultivated and wild  
461 lettuces are unambiguously European LeMoV isolates that tightly cluster with LeMoV  
462 sequences from South America (Figure 3A & B). The host range analysis identified a single  
463 consistent difference between LeMoV isolates and the JG1-A and JG1-B isolates, with the  
464 former being unable to infect *N. clevelandii* (Table 3). Considering together the phylogenetic  
465 analyses (Fig. 3), the species molecular criterion met for the Pol-Pro region, the differences in  
466 the predicted cleavage sites liberating the 3 CPs (Fig. 2 and Table 2) and the host range  
467 difference, we suggest that the JG1-A/B and 21-015C-A/B isolates could all be considered as  
468 belonging to a distinct species, for which the name lettuce star mosaic virus (LSMV) is  
469 proposed. LSMV is clearly pathogenic in lettuce, inducing star mosaic symptoms that may  
470 turn to pin-point or star necrosis and are quite similar to those caused by LeMoV, while more  
471 punctuated than the mosaics typically induced by lettuce mosaic virus (LMV). It should be  
472 noted that in the absence of fully conclusive diagnostics at the time, older reports of the  
473 presence of DaYMV in French lettuce crops (Blancard et al. 2003) might, in fact, have been  
474 related to LeMoV or LSMV.

475 Previous surveys of lettuce crops in France suggest that LSMV or LeMoV are rare, even  
476 though wild lettuce may possibly act as a reservoir in the absence of lettuce crops (hence the  
477 HTS analysis of the JG1 and JG2 plants following the observation of unusual symptoms).  
478 This situation is, at least for LeMoV, in contrast to that reported in the early 2000s in Brazil  
479 where LeMoV was considered the first or second most important lettuce virus, with a reported  
480 prevalence of 10.1%-26.1% under open field conditions in Sao Paulo state (Krause-Sakate et  
481 al. 2008; De Marchi et al. 2012). However, this situation changed during the last years, with  
482 LeMoV being now more rarely found and the main viral constraint now being thrips  
483 transmitted orthospoviruses (RKS, unpublished data). Identifying the causes underlying  
484 these differences epidemiological trends, which might be related to differences in agricultural

485 practices, varietal choice, insect vector populations or other yet unidentified factors will  
486 require further investigations. The availability of complete genomic sequences for LeMoV,  
487 LSMV and DaYMV, the characterization and genome sequencing of LWV1 and the RT-PCR  
488 assays developed here should provide useful tools for such studies and for a broader and more  
489 thorough estimation of the distribution, prevalence and impact of these viruses on lettuce  
490 crops worldwide.

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502

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TABLE 1. List of viral isolates detected in various cultivated and wild lettuces and in the RNA24P dandelion plant.

| Plant       | Virus          | Mapped reads | % Mapped reads | Average Coverage | BlastX best hit / (% of identity)     |
|-------------|----------------|--------------|----------------|------------------|---------------------------------------|
| JG1 (a)     | LWV1           | 26,980       | 33.7%          | 449x             | Bellflower vein chlorosis virus (49%) |
|             | LeCV1          | 12,327       | 15.4%          | 283x             | Carrot chordovirus 1 (54%)            |
|             | LSMV-JG1-A     | 4,198        | 5.2%           | 92x              | Parsnip yellow fleck virus (54%)      |
|             | LSMV-JG1-B     | 5,227        | 6.5%           | 102x             | Carrot necrotic dieback virus (47%)   |
| JG2 (b)     | LeMoV          | 119,027      | 0.38%          | 1,790x           | Carrot necrotic dieback virus (50%)   |
| P22 (a)     | LeMoV          | 2,517        | 29.3%          | 78x              | Parsnip yellow fleck virus (47%)      |
| LIB3 (a)    | LeMoV LIB3-A   | 800.864      | 29.3%          | 9,107x           | na                                    |
|             | LeMoV LIB3-B   | 365.279      | 13.2%          | 4,157x           | na                                    |
| 21-015C (b) | LeMoV          | 143,557      | 2.6%           | 2,024x           | na                                    |
|             | LSMV 21-015C-A | 33,503       | 0.6%           | 460x             | na                                    |
|             | LSMV 21-015C-B | 24,895       | 0.4%           | 343x             | na                                    |
| RNA24P (b)  | DaYMV-RNA24P-1 | 3,824        | 60.3%          | 60x              | Carrot necrotic dieback virus (49%)   |
|             | DaYMV-RNA24P-2 | 1,003        | 15.6%          | 15x              | Carrot necrotic dieback virus (49%)   |

For each viral contig, number of reads assigned to each isolate, the proportion of mapped reads and the average coverage are provided, together with information about the best BlastX hit. The results were obtained after HTS analysis of (a) double-stranded RNAs or (b) total RNAs from infected plants. LWV1: lettuce waikavirus 1, LeCV1: lettuce chordovirus 1, LSMV: lettuce star mosaic virus, LeMoV: lettuce mottle virus, DaYMV: dandelion yellow mosaic virus. na: does not apply

TABLE 2. Polyprotein dipeptides cleavage sites for viruses in the genera *Waikavirus* and *Sequivirus* and either (a) published or (b) predicted in the present work using a ClustalW-generated multiple alignment of the viral polyproteins.

| Virus sequences per species in this study | Hypothetical P1 / CP1 | CP1 / CP2     | CP2 / CP3       | CP3 / NTP-Protein Binding | NTP-Protein Binding / Pro <sup>#</sup> | Pro / RdRp <sup>#</sup> |
|---|-----------------------|---------------|-----------------|---------------------------|--|-------------------------|
| RTSV NC_001632 (a)                        | Q/A                   | Q/S           | Q/D             | Q/M                       | Q/D                                    | Q/A                     |
| MCDV NC_003626 (a)                        | Q/S                   | Q/M           | Q/V             | Q/M                       | Q/V                                    | S/P                     |
| BVCV NC_027915 (a)                        | Q/S                   | Q/S           | Q/G             | K/D                       | Q/D                                    | E/A                     |
| RcaV1 MH325329 (a)                        | Q/N                   | Q/N           | Q/L             | Q/M                       | Q/E                                    | A/A                     |
| BraV1 NC_040586 (b)                       | Q/S                   | Q/A           | Q/A             | Q/M                       | Q/N                                    | T/A                     |
| PWV LC_488189 (b)                         | Q/T                   | Q/A           | Q/S             | Q/K                       | Q/D                                    | S/V                     |
| LWV1 MT348710 (b)                         | Q/S (700/701)         | Q/S (922/923) | Q/S (1124/1125) | Q/T (1423/1424)           | Q/D (2536/2537)                        | Q/S (2873/2874)         |
| PYFV NC_003628 (a)                        | S/P                   | N/A           | Q/A             | Q/A                       | S/P                                    | Q/S                     |
| CNDV NC_038320 (b)                        | G/S                   | Q/A           | Q/S             | Q/G                       | ?                                      | ?                       |
| LeMoV-JG2 MT348707 (b)                    | E/P (385/386)         | Q/G (577/578) | Q/N (801/802)   | Q/A(1037/1038)            | ?                                      | ?                       |
| LeMoV-P22 MT348708 (b)                    | E/P                   | Q/G           | Q/N             | Q/A                       | ?                                      | ?                       |
| LeMoV-SP MT348709 (b)                     | E/P                   | Q/G           | Q/N             | Q/A                       | ?                                      | ?                       |
| LeMoV LIB3-A OQ446443 (b)                 | E/P                   | Q/G           | Q/N             | Q/A                       | ?                                      | ?                       |
| LeMoV LIB3-B OQ446444 (b)                 | E/P                   | Q/G           | Q/N             | Q/A                       | ?                                      | ?                       |
| LeMoV 21-015C OQ446445 (b)                | E/P                   | Q/G           | Q/N             | Q/A                       | ?                                      | ?                       |
| LSMV-JG1-A MT348705 (b)                   | N/P                   | Q/G           | Q/G             | Q/A                       | ?                                      | ?                       |
| LSMV-JG1-B MT348706 (b)                   | N/P (383/384)         | Q/G (575/576) | Q/G (799/800)   | Q/A (1035/1036)           | ?                                      | ?                       |
| LSMV-21-015C-A OQ446446 (b)               | N/P                   | Q/G           | Q/G             | Q/A                       | ?                                      | ?                       |
| LSMV-21-015C-B OQ446447 (b)               | D/P                   | Q/G           | Q/A             | Q/A                       | ?                                      | ?                       |
| DaYMV-RNA24P-1 MT559283 (b)               | K/P (388/389)         | Q/G (580/581) | Q/M (804/805)   | Q/G (1041/1042)           | ?                                      | ?                       |
| DaYMV-RNA24P-2 MT559284 (b)               | K/P                   | Q/G           | Q/S             | Q/G                       | ?                                      | -                       |

Amino acid positions of the cleavage sites on the polyprotein are provided in parentheses () for one representative isolate sequenced here of each species. #: a question mark indicates viruses for which it was not possible to identify a tentative dipeptide cleavage site.



TABLE 3. Infection and symptomatology of sequiviral isolates in various hosts following mechanical transmission.

|                                     | <b>JG1-AT348705</b> | <b>JG1-BT348706</b> | <b>LeMoV-JG2</b> | <b>LeMoV-<br/>AF197(a)</b> | <b>DaYMV(b)</b> |
|-------------------------------------|---------------------|---------------------|------------------|----------------------------|-----------------|
| <i>Chicorium endivia</i> cv. Anjou  | S (9/11)            | S (14/43)           | S (33/60)        | -                          | S               |
| <i>Lactuca sativa</i> cv. Trocadéro | S (22/24)           | S (13/18)           | S (29/32)        | S                          | S               |
| <i>Sonchus olearaceus</i>           | S (5/12)            | S (13/29)           | S (30/36)        | S                          | -               |
| <i>Nicotiana rustica</i>            | - (0/12)            | - (0/33)            | - (0/36)         | -                          | (ll)(s)         |
| <i>Nicotiana glutinosa</i>          | - (0/12)            | - (0/33)            | - (0/48)         | -                          | (ll)            |
| <i>Nicotiana benthamiana</i>        | s (6/12)            | s (27/36)           | s (29/36)        | ll, s                      | s               |
| <i>Nicotiana clevelandii</i>        | s (18/56)           | s (11/67)           | - (0/48)         | -                          | (ll)(s)         |
| <i>Chenopodium amaranticolor</i>    | - (0/11)            | - (0/32)            | - (0/48)         | -                          | LL, (S)         |
| <i>Chenopodium quinoa</i>           | - (0/12)            | S (7/32)            | - (0/36)         | LL, S                      | LL, (S)         |
| <i>Gomphrena globosa</i>            | s (1/11)            | s (11/55)           | s (14/56)        | LL, S                      | (LL), (s)       |

The number of infected over inoculated plants is indicated in parentheses. Also shown are infection and symptoms reported by Jadao et al. (2007) for lettuce mottle virus (LeMoV) isolate AF197 (a) and reported in Bos et al. (1983) for several isolates of dandelion yellow mosaic virus (DaYMV) (b). -, no infection; S, systemic symptoms; LL, local lesions; s, latent systemic infection; ll, latent local infection. Symptoms noted in parentheses reflect inter-isolate variability.

## LEGENDS TO THE FIGURES

**Fig. 1.** Symptoms caused by lettuce mottle virus (LeMoV) and lettuce star mosaic virus (LSMV) in various host plants. **A**, Necrotic mosaic symptoms observed on the original field-grown JG2 lettuce (cv. Guétary). **B**, Systemic necrotic lesions of LeMoV JG2 isolate on *Chicorium endivia* cv. Anjou 13 days post-inoculation (dpi). **C**, Systemic mosaic symptoms of LeMoV JG2 isolate on lettuce cv. Trocadéro at 13 dpi. The leaf on the left is from an uninoculated control plant. **D**, Symptoms of lettuce star mosaic virus (LSMV) isolate JG1-B on *Chenopodium quinoa* at 36 dpi. **E**, Symptoms of lettuce star mosaic virus (LSMV) isolate JG1-A on *C. endivia* cv. Anjou at 19 dpi. **F**, Symptoms of LSMV JG1-A (left) and JG1-B (right) in lettuce cv. Trocadéro.

**Fig. 2.** Schematic representation of the genomic organization drawn to scale of **A**, lettuce waikavirus 1 (LWV1) isolate JG1. **B**, lettuce star mosaic virus (LSMV) isolate JG1-B. **C**, lettuce mottle virus (LeMoV) isolate JG2. **D**, dandelion yellow mosaic virus (DaYMV) isolate RNA24P-1, partial. The short horizontal lines show the 5' and 3' untranslated regions, respectively. The large box is the long open reading frame with the coordinates of the beginning and the end mentioned. Predicted cleavage sites and their positions are shown as triangles under predicted mature proteins and uncertain cleavage sites by a question mark. P1, hypothetical protein 1; CP1, CP2, CP3, coat proteins 1, 2, and 3; NTB, NTP-binding helicase; PRO, 3C-like protease; RdRp, RNA-dependent RNA polymerase are indicated within the polyprotein. Predicted ORFX is indicated by a box for LWV1-JG1.

**Fig. 3.** Unrooted phylogenetic trees reconstructed using **A**, the Pro-Pol region, and **B**, the coat protein amino acid sequences from the members of the genera *Waikavirus* and *Sequivirus*, one representative species of other genera in family *Secoviridae*, and unassigned plant viruses showing sequence homology with the members of *Secoviridae*. The sequences of the viruses determined in the present work are indicated in bold font with black dots. The trees were constructed using the neighbor-joining method and the bootstrap values (500 replicates) greater than 70% are indicated next to the branches. The scale bar represents 10% aa divergence. The abbreviations and the accession numbers of the viruses used in this work are mentioned in Supplementary TABLE S4.

Figure 1

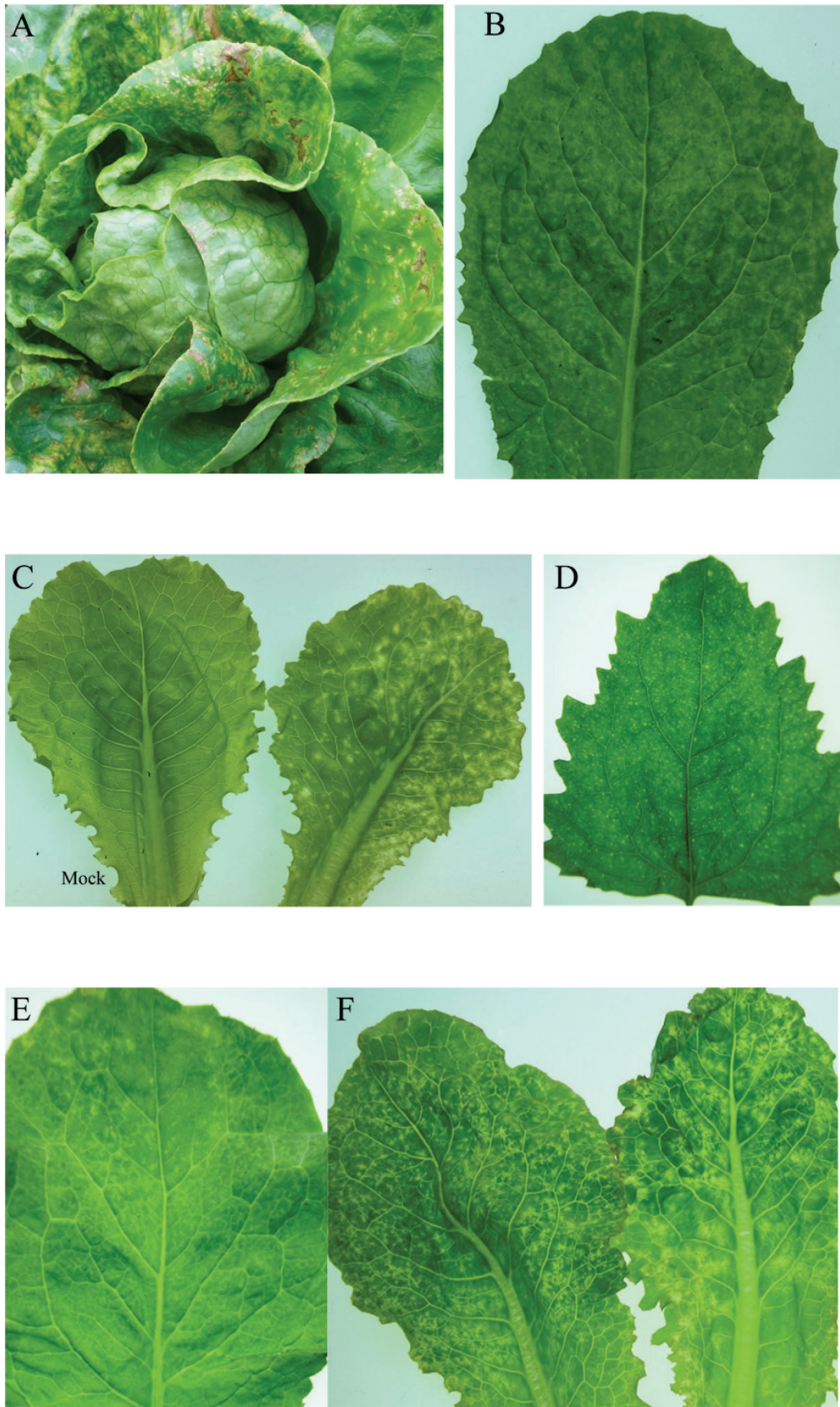
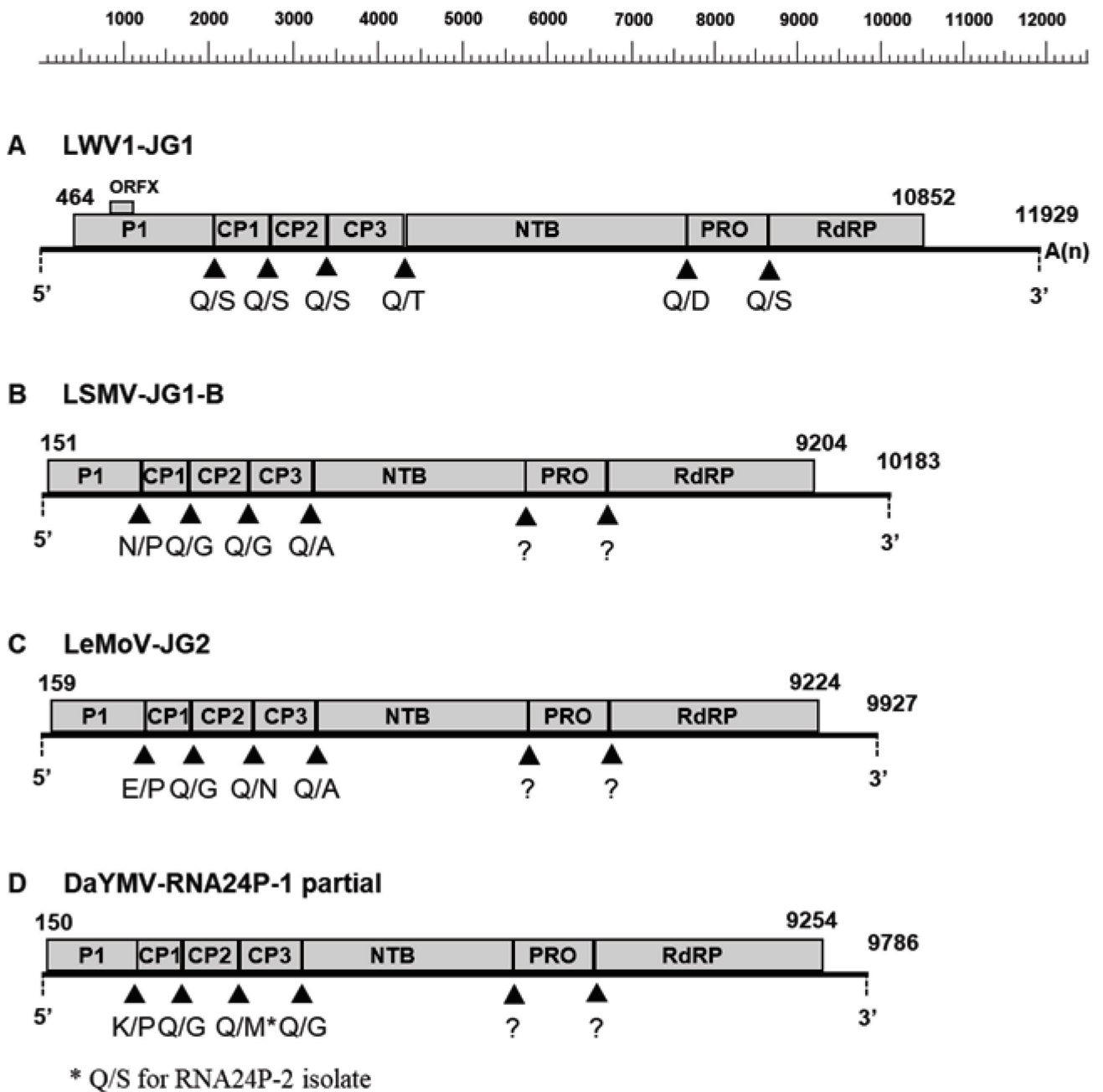
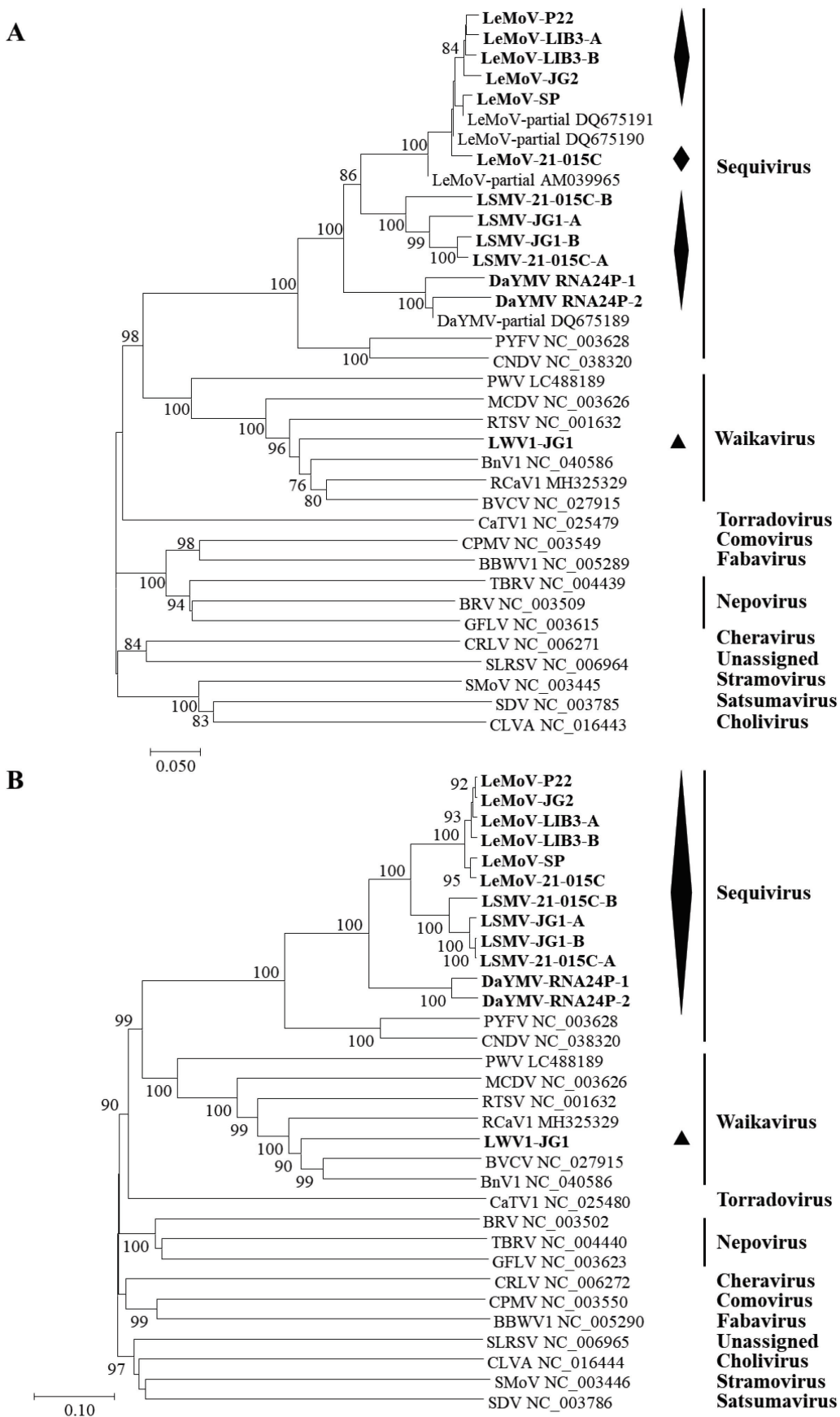


Figure 2



**Figure 3**



## **Supplementary table titles and Supplementary figure captions**

**Supplementary TABLE S1.** Primers used for the detection and the molecular characterization of lettuce mottle virus (LeMoV) isolates JG2, P22 and SP, of lettuce star mosaic virus (LSMV) isolates JG1-A and JG1-B and of lettuce waikavirus 1 (LWV1) isolate JG1.

**Supplementary TABLE S2.** Pairwise percentages of amino acid identity for the CP (lower diagonal) and Pro-Pol (upper diagonal) regions of the polyproteins of Waikavirus genus members.

**Supplementary TABLE S3.** Pairwise percentages of identity for the CP (lower diagonal) and Prot-Pol (upper diagonal) regions of the polyproteins of Sequivirus genus members.

**Supplementary TABLE S4.** Description of the viral isolates used for the phylogenetic studies

**Supplementary Figure 1.** Multiple alignment of the predicted short overlapping ORFX from all members of the genus Waikavirus.