

# 1 Complete sequence and genomic annotation of Carrot torradovirus

## 2 1 (CaTV1)

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### 11 Abstract

12 *Carrot torradovirus 1* (CaTV1) is a new member of the *Torradovirus* genus within the family  
13 *Secoviridae*. CaTV1 genome sequences were obtained from a previous Next Generation Sequencing  
14 (NGS) study and were compared to other members and tentative new members of the genus. The  
15 virus is comprised of a bipartite genome and RACE was used to amplify and sequence each end of  
16 RNA1 and RNA2. As a result RNA1 and RNA2 are estimated as containing 6944 and 4995  
17 nucleotides respectively, with RNA1 encoding the proteins involved in virus replication, and RNA2  
18 encoding the encapsidation and movement proteins. Sequence comparisons showed that CaTV1  
19 clustered within the non-tomato infecting torradoviruses and is most similar to *Motherwort yellow*  
20 *mottle virus* (MYMoV). The nucleotide identities of the Pro-Pol and coat protein regions were below  
21 the criteria established by the ICTV for demarcating species, confirming that CaTV1 should be  
22 classified as a new species within the *Torradovirus* genus.

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### 24 Introduction

25 *Carrot torradovirus 1* (CaTV1) was an incidental finding discovered in a Next Generation Sequencing  
26 (NGS) study seeking to elucidate the causes of internal necrosis in carrots (*Daucus carota*) in the UK

27 [1]. CaTV1 has been recently reported in carrot leaves collected in the Southwest of France,  
28 indicating its presence also outside the UK [2]. The virus is similar to members of the *Torradovirus*  
29 genus, first described in 2007 to place two new viruses affecting tomato crops, *Tomato torrado virus*  
30 (*ToTV*) and *Tomato marchitez virus* (*ToMarV*) [3, 4]. Later more viruses affecting different crops have  
31 been added to the genus, including, tomato chocolate virus (*ToChV*), Tomato chocolate spot virus  
32 (*ToChSV*), Tomato necrotic dwarf virus (*ToNDV*), *Lettuce necrotic leaf curl virus* (*LNLCV*), *Motherwort*  
33 *yellow mottle virus* (*MYMoV*), *Cassava torrado-like virus* (*CsTLV*) and *Squash chlorotic leaf spot virus*  
34 (*SCLSV*) [5-11]. *Torradoviruses* are considered members of the *Secoviridae* family within the order  
35 *Picornavirales* [12] and previous sequence comparisons within this genus established two different  
36 clades for tomato-infecting (TI) and non-tomato infecting (NTI) members [13].

37 Although CaTV1 was a sequencing finding using NGS, the sequence of the virus was not fully  
38 described. In this study we complete the characterization of the genome of CaTV1, including  
39 completion of the 3' and 5' ends of both RNA fragments using RACE, annotation of the genome  
40 identifying the location of translational features, and establishing the similarities between this and  
41 other members of the *Torradovirus* genus.

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### 43 **Completion of the CaTV1 genome**

44 The 3' and 5' ends of both RNA1 and RNA2 were amplified using the SMARTer RACE cDNA  
45 amplification kit (Clontech) according to the manufacturer's protocols. The 3' and 5' PCR products  
46 were analysed by direct sequencing. Results indicated that the 5' UTR and 3' UTR regions of RNA1  
47 were 127 and 240 nt long respectively, and for RNA2, were 611 nt (5' UTR) and 327 (3' UTR) in  
48 length. Completed sequences were deposited in GenBank with accession numbers KF533719.2 and  
49 KF533720.2 for RNA1 and RNA2 respectively.

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### 51 **CaTV1 genome organization**

52 CaTV1 - RNA1

53 As a member of the *Secoviridae* family, RNA1 is likely to code for a single ORF translated into a  
54 polyprotein which is then likely to be processed by serine-like proteases into mature proteins [12].  
55 Following analysis of RNA1, one predicted ORF (RNA1-ORF1) was identified (6944 nts), encoding a  
56 putative polyprotein of 2192 amino acids (aa) with a molecular mass of 249 kDa. The translational  
57 start (AUG) and stop (UAA) codons were found at nucleotide positions 127-129 and 6703-6705  
58 respectively (Figure 1).

59 The complete nucleotide sequence was compared to other sequences available in GenBank using  
60 BlastN, revealing 71 % identity to LNLCV (KC855266) and ToMarV (KT756874), 69 % to ToChSV  
61 (GQ305131) and ToTV (KM091449) and 68 % to MYMoV (KM855266) and ToNDV (KC999058), all  
62 members of the *Torradovirus* genus.

63 Identification of protein motifs were made based on previous characterization of ToTV and ToMarV [3,  
64 4]. The polyprotein contains the conserved Hel-Pro-Pol replication block typical of picorna-like viruses  
65 (nt positions 401-1516). Comparison of the aa sequence of the Pro-Pol region (1072-1516), limited  
66 by the “CG” motif of the 3C-like proteinase and the “GDD” motif of the RNA-dependent RNA  
67 polymerase (RdRp), suggested levels of similarity lower than those specified in the species  
68 demarcation criteria (< 80 %) established in the ninth ICTV report [14]. This conserved domain is  
69 typically used to determine differences among different picornavirales members. Pairwise  
70 comparisons using the Hel-Pro-Pol protein sequences with other torradoviruses, showed that CaTV1  
71 shares 58.5 %, 59.5 % and 41.2 % of the sequence with LNLCV, MYMoY and SCLSV respectively.  
72 Lower levels were found when the same region was compared to tomato-infecting torradoviruses.  
73 Typical helicase motifs (type III helicase), A (GKT), B (DD) and C (N) were found at positions 410, 456  
74 and 507 respectively. The region, between aa 401-508, shares levels of identity up to 84 % with the  
75 corresponding region of LNLCV and 82.9 % with MYMoV, while lower levels of identity were found in  
76 the tomato-infecting torradoviruses (46.4 % ToTV and 50 % ToMarV). A histidine residue in the  
77 putative protease substrate binding pocket is located at aa position 1075 and is required for  
78 proteolytic processing in members of the *Secoviridae* family. Typical RdRp motifs (I-VII) were found  
79 between aminoacid positions 1311-1596 [15]. Pairwise comparison revealed that the closest amino  
80 acid identities in the RdRp domain were found with LNLCV (75.1 %) and MYMoV and ToChV (70.5  
81 %).

82 To determine the relationship between CaTV1 and other viruses of the family, a neighbour-joining  
83 phylogenetic tree with 500 bootstrap replications was constructed with the RdRp region using MEGA6  
84 and ClustalX (figure 2a). The analysis revealed different clades for TI and NTI members confirming  
85 previous results described [13]. It also indicates differences between NTI torradoviruses in the RNA1  
86 with Squash chlorotic leaf spot virus (SCLSV), the latest proposed member of the genus, in an  
87 independent clade.

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#### 89 CaTV1 - RNA2

90 RNA2 (4995 nt) has two predicted ORFs encoding two polyproteins. ORF1 encodes a putative  
91 polyprotein of 202 aa with a predicted molecular weight of 22 kDa (figure 1). This ORF, partially  
92 overlaps the large ORF2, and is a distinguishing feature of the *Torradovirus* genus. The closest  
93 related member was MYMoV (83 % identity). No conserved motifs were found in the sequence and  
94 several differences were seen between TI and NTI torradoviruses, confirming previous results  
95 reported [13].

96 ORF2 encodes a large polyprotein of 1167 aa (130 kDa). The translational start (AUG) codon was  
97 found at nt positions 1165-1167 while the stop (UAA) codon was at nt positions 4666-4668. When the  
98 whole nucleotide sequence was compared to other members of the *Secoviridae* family, the most  
99 closely related members were LNLCV (68 % identity) and MYMoV (66 % identity).

100 The region encoding the movement protein (MP) domain was found between aa 45-239 with the  
101 typical LxxPxL motif in positions 211-216. Based on homology to related viruses and previous  
102 identification of ToMarV and ToChSV cleavage sites [16], the cleavage site between the MP and the  
103 first coat protein is likely to be located at position 487 (Q<sup>487</sup>/A<sup>488</sup>). The coat protein region shares  
104 levels of identity of 42.2 % and 47.3 % with LNLCV and MYMoV respectively, which again are the  
105 closest related members. This percentage also demarcates CaTV1 as a new species of the genus  
106 according to the criteria established in the ICTV ninth report [14]. A glutamine residue (Q) at position -  
107 1 is highly conserved in all the torradoviruses and could potentially act as a protease cleavage site  
108 among the different proteins [16]. By comparison with ToMarV and ToChSV cleavage sites, a Q  
109 residue has been identified at position 695 (Q<sup>695</sup>/S<sup>696</sup>) which could be a potential cleavage site

110 between the Vp35/Vp26 coat proteins. Additionally, the putative Vp26 and Vp23 cleavage site region  
111 would be located at aa position 935 (Q<sup>935</sup>/I<sup>936</sup>). However, the actual number and size of the predicted  
112 capsid proteins for CaTV1 have not been determined experimentally.

113 A phylogenetic tree was constructed with all the members of the *Torradovirus* genus using the whole  
114 region with the three coat proteins (Figure 2b). In agreement with the results obtained with RNA1,  
115 three different clades can be again differentiated: five viruses cluster in the tomato-infecting  
116 torradovirus clade; CaTV1 is grouped with MYMoV and LNLCV in the non-tomato infecting (NTI)  
117 torradovirus clade; and SCLSV clusters with Cassava torrado-like virus (CsTLV) sequences in a third  
118 independent clade.

119 CaTV1 is transmitted by aphids [17] and a search of possible aphid transmission motifs was carried  
120 out comparing the coat protein region of TI and NTI torradoviruses. DAG (Asp-Ala-Gly) is usually a  
121 highly conserved motif in N-terminal of the coat protein of potyviruses and it has been demonstrated  
122 that the change of any of the amino acid prevents transmission by aphids [18]. However this motif  
123 could not be found within the CaTV1 sequence. TI torradoviruses have been described to be whitefly-  
124 transmitted [19, 20], but no transmission motifs have been identified in the sequences for any of these  
125 members so far.

126 The presence of a small (3–5 kDa) VPg linked to the 5' end of the RNAs has been confirmed for most  
127 members of the order; comparative genomics strongly suggests that this property is universally  
128 conserved among the genus and the *Secoviridae* family [13].

129 This study has described a complete characterization of the whole genome sequences of CaTV1 for  
130 both RNAs by comparison with other members of the genus and confirms that CaTV1 is a new  
131 species according to the criteria established by the ICTV. Phylogenetic studies using both RNAs have  
132 also confirmed and given further evidence of the differences between non-tomato infecting  
133 torradoviruses.

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135 **Compliance with ethical standards**

136 This research was supported as project FV 382b through UK government funding under Defra Plant  
137 Health Capability. All authors declare that they have no conflict of interest. This article does not  
138 contain any studies with human participants or animals performed by any of the authors

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225 Figure legends

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227 **Figure 1:** Proposed genetic diagram for CaTV1 RNA1 and RNA2 with the positions of the ORFs  
228 noted. Relative positions of regions containing helicase, protease and RNA-dependent RNA  
229 polymerase motifs on RNA1, and movement protein and coat proteins on RNA2 are indicated.

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231 **Figure 2:** (A) A phylogenetic tree of sequences of viruses within the *Secoviridae* family was  
232 constructed using the RdRp amino acid region. (B) A second phylogenetic tree constructed using the  
233 whole coat protein region of CaTV1 RNA2 of all the members of the *Torradovirus* genus. Alignments  
234 were done using MEGA6 using the neighbour-joining algorithm. Sequences of all the viruses were  
235 chosen from GenBank and accession numbers are shown. The numbers at the branch points are the  
236 percentage bootstrap values following 500 bootstrap resampling and the scale indicates the number  
237 of amino acid substitutions per site.

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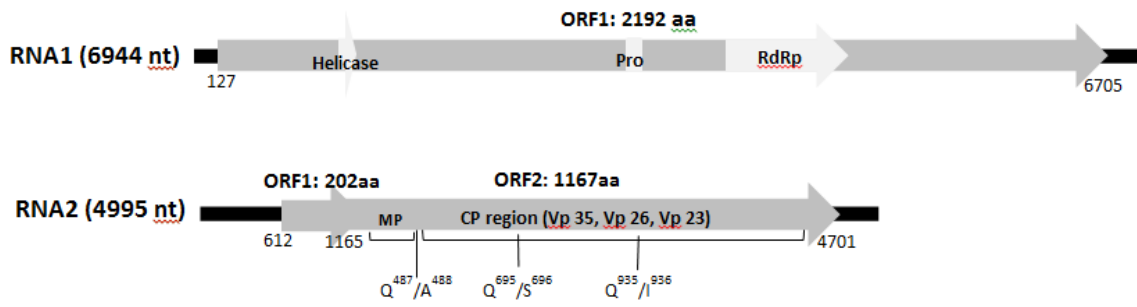
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251 Figure 1

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269 **Figure 2**

