



First report of *Turnip crinkle virus* infecting garlic mustard (*Alliaria petiolata*) in Germany

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In May 2018, three samples of wild garlic mustard (*Alliaria petiolata*, Brassicaceae) were collected from a private garden in Koenigslutter, Germany. While sample EPV_18_002 was asymptomatic apart from slight yellowing, samples EPV_18_003 and EPV_18_004 showed stunting, yellowing, necrosis and severe crinkling (Figs. 1-3). It was possible to mechanically transmit the suspected virus from all three samples to *Nicotiana benthamiana*; the same systemic symptoms of leaf crinkling, rolling and yellowing appeared seven days post infection on all plants, and the plants died in the second week. Infected *N. benthamiana* leaves were analysed by electron microscopy and icosahedral particles of c. 30 nm in diameter were observed that reacted with antibodies (Julius Kühn Institute, reference number JKI-1177) raised against a UK isolate of *Turnip crinkle virus* (TCV) (Fig. 4).

To obtain the full viral genome, dsRNA was extracted from symptomatic *N. benthamiana* leaves (inoculated from sample EPV_18_002) using a Viral dsRNA Extraction Mini Kit for Plant Tissue (iNtRON, South Korea) and sent for library preparation and sequencing on Illumina NovaSeq 6000 platform (2x150 bp) at Eurofins GATC Biotech GmbH, Germany. Using Geneious Prime (2019.0.4), the raw reads were quality trimmed, filtered, error corrected and normalised, followed by *de novo* assembly. A BLASTn search of the generated contigs confirmed the presence of eight contigs (131 to 4,057nt) with nucleotide (nt) identities of 82.3% to 86.7% to TCV (NC_003821); there was no indication of the presence of satellite RNAs. The full genome of the German TCV isolate (JKI ID 29306) was assembled using this reference genome. The complete genome of TCV-JKI-29306 was 4,061 nt (accession no. MK301398). The presence of TCV was confirmed in all three original samples by RT-PCR using OneTaq One-Step RT-PCR Kit (NEB, USA) with the primer pair (HZ632 5'-AAAGGCAAACTGGGTGGGA-3' and HZ633 5'-TAAAGTTTGGCGCTAGGGG-3') generating a 339 bp fragment.

In further comparisons using MUSCLE (3.8.425, Edgar, 2004), the German TCV isolate shared 82.8% nt identity to NC_003821 and 82.6% to AY312063 (Table 1). Additionally, the protein sequences of the different

TCV genes shared identities of 75.0 to 90.7% to the proteins of NC_003821 and AY312063 (Table 1). According to ICTV criteria, these data indicate the presence of a novel TCV strain (Rochon *et al.*, 2012).

TCV belongs to the genus *Betacarmovirus* (family *Tombusviridae*). It infects members of the *Brassicaceae* family causing crop losses (Broadbent & Heathcote, 1958; Lister, 1958). To our knowledge, this is the first report of a TCV strain from Germany occurring on garlic mustard. Although TCV is one of the model organisms in plant virology and therefore well studied, there is surprisingly little literature available on occurrence, host range and impact of this virus. As the original host plants appeared as weeds in a private garden (approximately 40 plants with a minority displaying symptoms), the impact of TCV in Germany and other countries on cultivated crop plants is currently unknown.

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Figure 1



Figure 2



Figure 3

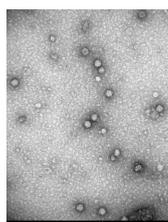


Figure 4

Table 1. Pairwise identities of the *Turnip crinkle virus* (TCV) strain from garlic mustard in Germany with whole TCV genomes from the UK using a multiple sequence alignment tool, MUSCLE.

Accession no.	Genome nucleotide identity (%)	Amino acid identity (%)				
		p88 RP	p28 ARP	p8 MP	p9 MP	p38 CP
NC_003821	82.8	90.5	84.4	75.0	89.4	85.5
AY312063	82.6	90.7	84.8	76.4	88.2	85.2

Figure 5

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