*Article*

# Molecular characterization of divergent closterovirus isolates infecting *Ribes* species

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**Abstract:** Five isolates of a new putative member of the genus *Closterovirus*, tentatively named blackcurrant leafroll associated virus 1 (BcLRaV-1), were identified in currant. The 17 kb long genome of BcLRaV-1 contained 10 open reading frames (ORFs). The replication associated polyprotein has two papain-like leader proteases, a methyltransferase, a helicase and an RNA-dependent RNA polymerase domain. Additional ORFs coded for heat shock protein 70 homolog, heat shock protein 90 homolog, two divergent copies of coat protein, and three accessory proteins without identifiable functions. Phylogenetic analysis showed that BcLRaV-1 is related to members of the genus *Closterovirus* and recombination analysis of the isolates showed clear evidences of intraspecies recombination.

**Keywords:** *Ribes*; currant; closterovirus; recombinants/recombination

## 1. Introduction

Black and red currants (*Ribes* spp.) are economically important berry crops. They are deciduous, unarmed shrubs native to northern latitudes of Asia, Europe and North America and belong to *Coreosma* and *Ribesia* subgenera of the genus *Ribes* [1]. The genus is diverse and includes more than 150 diploid species and numerous cultivated varieties [2]. Studies of virus and virus-like diseases in currants started in early 20th century and include reports of alfalfa mosaic virus, arabis mosaic virus, cucumber mosaic virus, raspberry ringspot virus, strawberry latent ringspot virus, tomato ringspot virus, tobacco rattle virus, gooseberry vein banding virus, black currant reversion virus and black currant yellows, interveinal white mosaic, and yellow leaf spot disease [2-4]. New currant viruses have recently been identified using traditional methods or high-throughput sequencing (HTS) [5-10].

Notwithstanding the progress in currant virology there are still gaps in knowledge, one of which is addressed here through the characterization of a new closterovirus complex affecting both black and red currant. Roberts and Jones observed closterovirus-like particles in *Ribes* already in 1997. Besse et al. (2010) observed similar particles in currants showing downward rolling of leaves and interveinal reddening in summer and autumn [5]. They produced antisera for serological detection and designed primers allowing for detection of two molecular variants of this virus. In 2015, Ho *et al*.

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reported a closterovirus in black currant in USA and developed a molecular diagnostic assay for it [11].

The *Closteroviridae* is a family comprised of non-enveloped viruses with one of the largest genomes among plant viruses with positive sense single-stranded RNA genomes. Currently the family includes the genera *Ampelovirus*, *Closterovirus*, *Crinivirus* and *Velarivirus,* with vectors ranging from mealybugs and soft scales to aphids and whiteflies [13]. Genomic segments are encapsidated in characteristic long flexuous particles consisting of major (CP) and minor (CPm) capsid proteins [12]. A genomic signature of closterovirids is the presence of a five-gene block of proteins involved in virion assembly and movement that, in addition to two CPs, include a small transmembrane p6 protein, the ~60 kDa protein, and a HSP70h, a homolog of the class of heat-shock proteins [13]. The host range of closteroviruses is usually narrow whereas acquisition of multiple non-conserved accessory genes is believed to play a role in host range expansion [13].

Here, we analyzed in depth a novel closterovirus species, tentatively named 'blackcurrant leafroll associated virus 1' (BcLRaV-1), identified in black and red currant, characterized its genomic organization, phylogeny, particle morphology and identified possible intraspecies recombination events.

## 2. Materials and Methods

### *2.1 Transmission electron microscopy.*

Purified virus particles of a isolate BC28074 were observed with a Tecnai F-20 transmission electron microscope, as described by Gugerli and Ramel [14].

### *2.2 Genome assembly and organization.*

The genome sequencing of all BcLRaV-1 isolates was done independently by four labs and the data were deposited in GenBank database (Table 1).

**Table 1.** Origin of currant isolates and description of HTS

| BcLRaV-1 isolate, GenBank accession number | Plant | Origin/Sequenced by | Symptoms, references | Sequencing and analyses | | |
|---|---|---|---|---|---|---|
| | | | | Input | Method | HTS Output |
| G55, MH460557 | Red currant, Gabreta 55 | Czech Republic | no obvious symptoms | Total RNA after mRNA enrichment | HTS, Sanger | 20 mil, 100 bp reads |
| GR, MH460558 | Red currant, Gondouin Rouge | Czech Republic | no obvious symptoms | | | |
| SLO, MH480582 | Black currant | Slovenia | no obvious symptoms | Ribo depleted total RNA | | 10 mil, 2x150 nt reads |
| BC28074, *AN will be added during review process* | Black currant, 28074 | Switzerland | Leafroll-like symptoms [5] | Viral associated nucleic acid (VANA) | | 50 mil, 2x75 nt reads |
| US, *AN will be added during review process* | Black currant | USA NCGR accession PI 556169 Unknown cultivar | Yellow line-pattern symptoms [11] | Double stranded RNA enrichment | | 454 Junior, 76,214 reads |

Both BcLRaV-1-GR and -G55 isolates were sequenced in two steps. Firstly, a composite sample containing total RNA extracted with GeneJET Plant RNA Purification Kit (Thermo Fisher Scientific, Vilnius, Lithuania) from four different red currant accessions was subjected to HTS (SeqMe s.r.o., Dobříš, Czech Republic), which included an intermediate mRNA enrichment step (TruSeq Stranded mRNA kit, Illumina, USA). The sequence verification and gap filling were done with Sanger sequencing of either PCR amplicons obtained with Sapphire Mastermix (Takara, Kusatsu, Japan) or cloned into pGEM T-Easy vector system (Promega, USA). PCR fragments larger than 2 kb were amplified with Q5 High-Fidelity Master Mix (NEB, USA). The 5' termini were completed and sequenced with a 5' RACE kit (Invitrogen, Carlsbad, CA, USA) and the 3' ends were derived as described earlier [15].

The genome of the BcLRaV-1-US isolate was obtained by a combination of HTS and Sanger sequencing. HTS was performed on degenerate oligonucleotide-primed reverse transcription-PCR (DOP RT-PCR) products derived from double-stranded RNA enriched (dsRNA) material of the infected plant following the procedures described previously [6]. The regions that were not covered by HTS were obtained by RT-PCR reactions using virus specific primers. The 5' terminal sequences were obtained using FirstChoice RLM-RACE Kit (Thermo Fisher Scientific) while the 3' ends were obtained using RACE-RT-PCR on polyadenylated RNAs (Poly (A) Tailing Kit, Applied Biosystems, USA). All PCR products were sequenced to get at least three-fold coverage of the regions.

The BcLRaV-1-BC28074 isolate was sequenced using virion-associated nucleic acids (VANA) approach. Virus particles were purified from mature leaves as previously described [16]. Then, viral RNA was extracted from purified viruses using RNeasy Plant Mini kit (Qiagen, Germany). Total RNA librairy was then prepared following TrueSeq Stranded mRNA kit (Illumina) and used for sequencing on an HiSeq 4000 by Fasteris SA (Switzerland). 5′ and 3′ terminal sequences of BC28074 were obtained using RACE System for Rapid Amplification of cDNA Ends (Invitrogen). At least two PCR amplicons were cloned and Sanger sequenced.

For the BcLRaV-1-SLO isolate, total RNA was extracted from 100 mg of leaf tissue using RNeasy Plant Mini Kit (Qiagen, Sverige, Denmark) where RLT buffer was supplemented with 10% Plant RNA Isolation Aid (Thermo Fisher Scientific). The extracted total RNA was quantified on Bioanalyzer 2000. Ribosomal RNA was depleted using RiboMinus Plant Kit for RNA-Seq (Thermo Fisher Scientific) and total RNA libraries were then prepared following TrueSeq Stranded mRNA kit (Illumina) without the enrichment step of poly-A. Libraries were sequenced on a Nextseq 500 sequencing machine at Liege University in Belgium with a read length of 2x150 nt. (Etiology fair COST Divas). For Sanger sequencing, the PCR amplification was done using Phusion Flash High Fidelity Master Mix (Thermo Fisher Scientific) and PCR products were directly sequenced (Macrogen, Korea).

### *2.3 In-silico analyses*

Sequence analyses were done in CLC Genomics Workbench 9.5.1 (Qiagen) and Geneious 9.1.5 (Biomatters Limited, Auckland, New Zealand). Multiple sequence alignments were built with MAFFT [17]. Phylogeny reconstructions were inferred using Maximum likelihood method with Approximate likelihood ratio branching testing. The phylogenetic trees were visualized using Interactive Tree Of Life v3 tool [18]. Putative recombination events were detected and evaluated in a program RDP4 [19].

## 3. Results and discussion

### *3.1. Transmission electron microscopy*

After particle purification of BcLRaV-1-BC28074-positive leaf material with leafroll symptoms (Fig. 1a), long thread-like particles were visualized (Fig. 1b) that are typical for members of the family *Closteroviridae*, with the most frequent length of 1,500 nm and width of ca. 11 nm ($n$ = 125).

(a) (b)

**Figure 1. (a)** Leafroll symptoms on the black currant plant 28074: downward curling of leaf margins and intervenal red coloration (Switzerland, July 2017); **(b)** Individual particles obtained after viral particles enrichment of the black currant 28074 leaf material, the scale bar represents 200 nm.

*3.2. Nucleotide sequence and genome organization of BcLRaV-1*

Using both HTS and Sanger sequencing, the genomes of five isolates from Europe and North America, found in both black and red currants, were reconstructed (Table 1).

The genome length ranges from 16,996 to 17,313 nucleotides (nt) and codes for 10 ORFs (Fig. 2 and Table 2). The results of 3' RACE with virus-specific primers on BcLRaV-1-GR and -G55 suggested an absence of poly(A) tail at the 3' terminus, similarly to other closteroviruses.

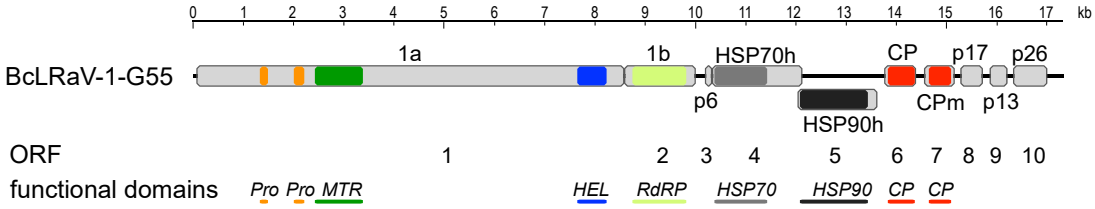


**Figure 2.** Schematic representation of the genomic organization of the isolate G55 of blackcurrant leafroll associated virus 1 (BcLRaV-1-G55). The genome is drawn as a black line and predicted ORFs as shaded rectangles. Annotations, ORF numbers and identified functional domains, are given below. Abbreviations: Pro – papain-like leader proteinase, MTR – methyltransferase, HEL – helicase, RdRP - RNA-dependent RNA polymerase, HSP70 - heat shock protein 70, HSP90 - heat shock protein 90, CP – capsid protein, CPm – minor capsid protein.

The genomic organization of all BcLRaV-1 isolates is identical (Fig. 2). There is a core of five genes with accessory three genes at the 3' genome part. The latter encode proteins of the BcLRaV-1 with no identifiable orthologs in other organisms. This is typical for all closteroviruses, whose genomes contain a number of proteins with unknown functions [20].

**Table 2.** Genome characteristics of BcLRaV-1 isolates

| Region | Protein Name | Length, nt | Function | TM | Mr, kDa |
|---|---|---|---|---|---|
| Genome | NA | 16,996-17,313 | NA | NA | NA |
| 5′ UTR | NA | 97-102 | NA | NA | NA |
| 3′UTR | NA | 264-309 | NA | NA | NA |
| ORFs 1-2 | 1a/1b | 9,906-9,942 | Replication | Yes | 370.3-372.8 |
| ORF3 | p6 | 147-150 | Movement | Yes | 5.6-5.7 |
| ORF4 | HSP70h | 1,797 | Movement | No | 65.5-65.9 |
| ORF5 | HSP90h | 1,590-1,593 | Virion assembly | No | 60.4-61.0 |
| ORF6 | CP | 636 | Encapsidation | No | 23.4-23.6 |
| ORF7 | CPm | 606 | Encapsidation, virion assembly | No | 22.3-22.5 |
| ORF8 | p17 | 441-456 | Unknown | No | 16.4-16.8 |
| ORF9 | p13 | 348-399 | Unknown | No | 13.2-15.0 |
| ORF10 | p26 | 687-690 | Unknown | No | 25.5-25.8 |

NA – not applicable, TM – transmembrane domain, predicted with TMHMM 2.0c

ORFs 1 and 2 encode the replication-associated proteins (Table 2) in which ORF2 is presumably translated by a +1 ribosomal frameshift from ORF1, a mechanism prevalent in closteroviruses [20]; resulting in a fusion polyprotein 1a/1b. The sequence surrounding the potential ribosome +1 slippage site is conserved in all isolates: cg(a/g/c)guuUAAcua (stop codon of the ORF1 is in capitals, the first proposed codon of ORF2 is underlined). Conserved domain search identified five replication-associated domains in the 1a/1b protein (Fig. 2). Two copies of a papain-like leader proteinase (Pro; pfam05533) were found upstream of methyltransferase motif (MTR; pfam01660). The copies are quite diverse, sharing only from 21 % to 30 % aa identity within each isolate. While intra-genome duplication of coat proteins is a fairly common feature of the family members, two copies of the leader protease are only present in some members of the genus *Closterovirus*. It was suggested that duplication events were independent in different species which was followed by functional divergence of each copy [21]. The roles of previously studied viral leader proteases are not limited to self-processing (proteolysis) but also include regulation of genome replication and transcription [22]. Host-specific effects were demonstrated for leader proteinases of grapevine leafroll-associated virus-2 (GLRaV-2) and particularly suggested that such diversification is needed for a closterovirus infection of perennial and/or woody plants [22].

The C-proximal part of the 1a/1b protein contains a viral helicase (HEL; Superfamily 1, pfam01443) and an RNA-dependent RNA polymerase (RdRP; pfam00978). Together with the MTR domain located at the 1a/1b N-terminus they constitute a replication module conserved within the entire alphavirus superfamily [23]. In other closteroviruses, a large region between MTR and HEL domains is believed to be either cleaved by an unidentified viral or cellular protease [24]. The putative ORF3, encodes a p6 protein predicted to contain a transmembrane domain (Table 2). The p6 counterpart in beet yellows virus (BYV) is associated with the endoplasmic reticulum and functions as a cell-to-cell movement protein [25]. It is separated by a short intergenic region from the putative heat shock protein 70 homologue (HSP70h; cd10170). The HSP70h protein of BYV and other closteroviruses is an integral part of the virion and plays a role in cell-to-cell movement through its ATPase activity [26]. The ORF5, coding for a HSP90h (pfam03225), partially overlaps the 3'-proximal part of ORF4. Two putative structural proteins, the major and minor capsid proteins (CP and CPm, respectively; (pfam01785)), are encoded by ORF6 and 7. The closteroviral CPm was shown to be essential for encapsidation of the 5' part of the viral RNA. The three predicted ORFs downstream of capsid proteins (p17, p13, and p26) did not have significant (E value cutoff $10^{-3}$) similarity with other viral proteins. Noticeably, the p26 protein shows higher conservation than either p17 or p13 among the isolates (61 % - 99 % Fig. 3). None of the three proteins were predicted to contain transmembrane domains (Table 2). Downstream of capsid proteins closteroviruses encode a variable number of

accessory proteins and their functionality was determined only for some. For example, p20 and p21 proteins of BYV participate in systemic transport and suppression of RNA silencing, respectively [20]. In citrus tristeza virus (CTV), an additional suppressor, p23 protein, was identified without any identifiable orthologs in other closteroviruses [27].

*3.3. Divergence of BcLRaV-1*

Nucleotide divergence between the BcLRaV-1 isolates was high and reached 39 % (Fig. 3). Black and red currant isolates separately showed higher nucleotide identities, 65 % and 71 %, respectively. For individual proteins, average identities varied from 45 % for putative p13 to 83 % for the HSP70h.

| Pair | Genome, nt identity, % | Gene, aa identity, % | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 1a/1b | p6 | HSP70h | HSP90 | CP | CPm | p17 | p13 | p26 |
| SLO vs US | 98 | 99 | 96 | 99 | 98 | 98 | 98 | 99 | 92 | 99 |
| BC28074 vs US | 65 | 63 | 63 | 84 | 78 | 85 | 80 | 61 | 47 | 76 |
| SLO vs BC28074 | 64 | 64 | 78 | 84 | 78 | 85 | 80 | 61 | 45 | 76 |
| SLO vs G55 | 61 | 62 | 71 | 79 | 66 | 78 | 78 | 52 | 32 | 62 |
| SLO vs GR | 61 | 62 | 78 | 79 | 67 | 76 | 77 | 51 | 31 | 63 |
| GR vs US | 61 | 62 | 73 | 79 | 67 | 76 | 78 | 51 | 31 | 62 |
| GR vs BC28074 | 61 | 63 | 71 | 78 | 69 | 76 | 77 | 51 | 30 | 61 |
| G55 vs US | 61 | 62 | 67 | 79 | 66 | 78 | 77 | 52 | 31 | 62 |
| G55 vs BC28074 | 61 | 63 | 76 | 78 | 68 | 78 | 76 | 51 | 30 | 61 |
| G55 vs GR | 72 | 67 | 85 | 95 | 90 | 93 | 94 | 80 | 77 | 82 |
| *average* | *67* | *67* | *76* | *83* | *75* | *82* | *82* | *61* | *45* | *70* |

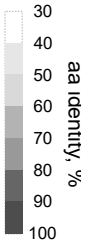


**Figure 3.** Pairwise whole genome nucleotide and predicted amino acid protein identities between the BcLRaV-1 isolates.

Noticeably, neither of the predicted 1b, HSP70h, and CP proteins was more than 25 % divergent, the species demarcation identity criterion set up for closteroviruses. Any two isolates, except SLO and US, did not share more than 90 % of amino acid identity in all genes. The isolates infecting red currant were more than 80 % identical with the exception of p13 protein. Both CP and CPm had similar levels of identity among the BcLRaV-1 isolates (Fig. 3).

The genome identity of the BcLRaV-1 isolates resembles those of grapevine leafroll-associated viruses 3 and 4, members of the genus *Ampelovirus* [28,28] with values of 62 % and 68 %, respectively. For members of *Closterovirus* genus, the most distant examples could be found among CTV and GLRaV-2, whose isolates share 79 % and 72 % of overall nt identities, respectively. Further analysis of CPs amino acids homology among different isolates of CTV and GLRaV-2 revealed the extent of divergence comparable to BcLRaV-1 (Fig. 4).

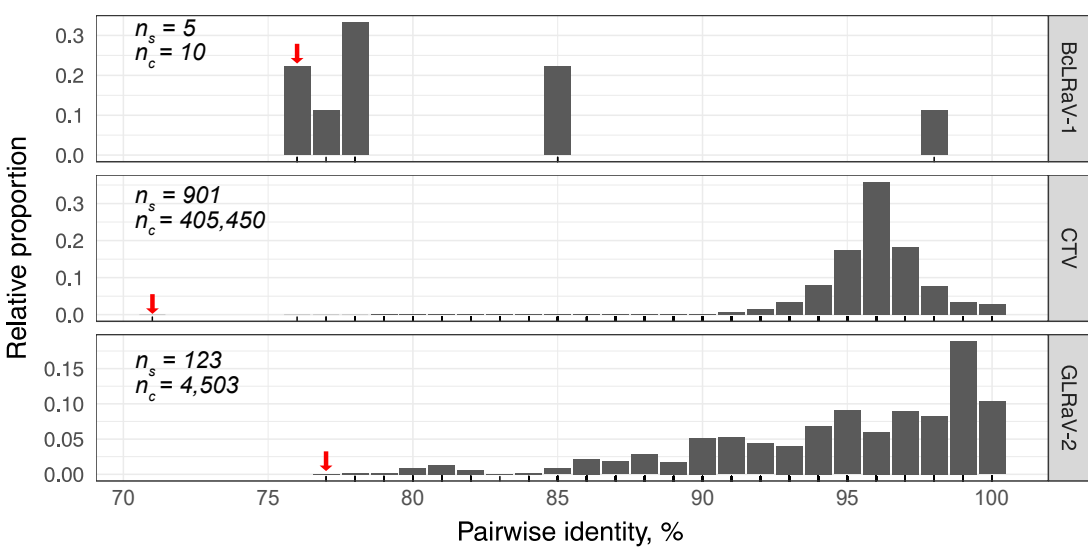


**Figure 4.** Distribution of pairwise amino acids CPs sequence homologies of BcLRaV-1, *Grapevine leafroll-associated virus 2* (GLRaV-2) and *Citrus tristeza virus* (CTV). Complete sequences were downloaded from the GenBank database (June 2018). Number of analyzed sequences (ns) and their pairwise combinations (nc) are shown in the left upper corner of each plot. The lowest identity value is annotated with a red arrow. Additionally, positions of the data points along *x* axes are denoted by tick marks.

Interestingly, 5' as well as 3' UTRs of BcLRaV-1 isolates showed considerable divergence with 65 % – 81 % and 56 % – 76 % of conserved positions, respectively. For example, some CTV isolates were showing only 60–70 % of nt identity in 5' UTRs, which despite that were predicted to form similar secondary structures [30].

*3.4. Phylogenetic analysis*

Maximum likelihood phylogeny inference of amino acid sequences of 1b (RdRp) and CP of the five isolates and representative members of the family confirmed taxonomical status of BcLRaV-1 in the *Closterovirus* genus (Fig. 5). Interestingly, the phylogenetic tree based on the RdRp and CP sequences (Fig. 5a, b) showed clear separation of BcLRaV-1 from members of the genus, whereas analysis based on the HSP70h sequences (Fig. 5c) supported its clustering with strawberry chlorotic fleck-associated virus, raspberry leaf mottle virus, rose leaf rosette-associated virus, and CTV. Further, topology of branching of the BcLRaV-1 isolates showed some discrepancy in both cases when the black currant isolates clustered together in the CP tree (Fig. 5b) but were separated in the 1b tree (Fig. 5a) This may be a result of occurred recombination. To test this hypothesis, a recombination analysis of BcLRaV-1 isolates and related closteroviruses was performed.

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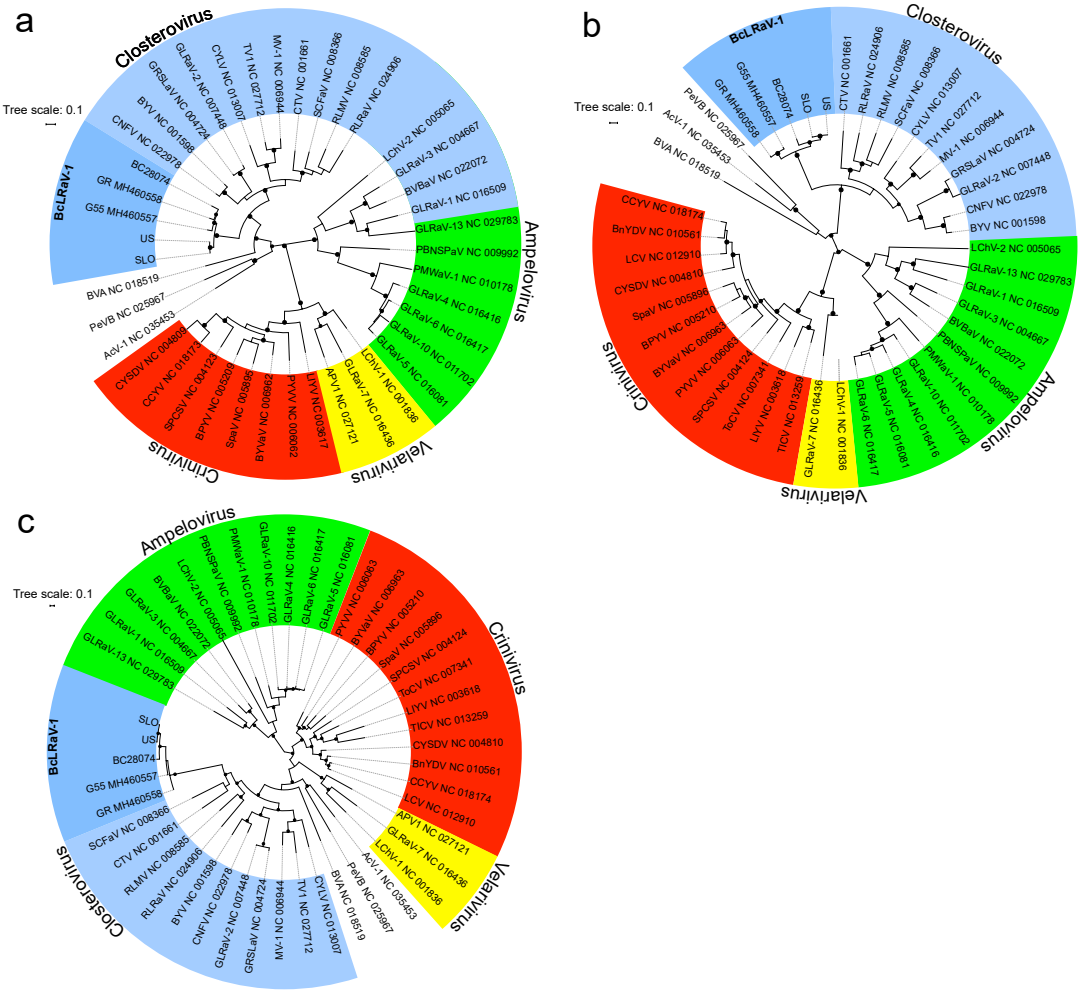


**Figure 5.** Phylogenetic analysis of aligned amino acid sequences of the (a) replicase (ORF 2) (b) the coat protein (ORF6) and (c) HSP70h (ORF4). Branching support with values more than 80% is indicated by dots. Amino acid sequences of the following members of the family *Closteroviridae* used in the analysis were obtained from GenBank: actinidia virus 1 (AcV-1), areca palm velarivirus 1 (APV1), bean yellow disorder virus (BnYDV), beet pseudoyellows virus (BPYV), beet yellows virus (BYV), blackberry vein banding-associated virus (BVBaV), blackberry yellow vein-associated virus (BYVaV), blueberry virus A (BVA), carnation yellow fleck virus (CNFV), carrot yellow leaf virus (CYLV), cucurbit chlorotic yellows virus (CCYV), Cucurbit yellow stunting disorder virus (CYSDV), grapevine leafroll-associated virus 1 (GLRaV-1), grapevine leafroll-associated virus 3 (GLRaV-3), grapevine leafroll-associated virus 4 (GLRaV-4), grapevine leafroll-associated virus 5 (GLRaV-5), grapevine leafroll-associated virus 6 (GLRaV-6), grapevine leafroll-associated virus 7 (GLRaV-7), grapevine leafroll-associated virus 10 (GLRaV-10), grapevine leafroll-associated virus 13 (GLRaV-13), grapevine rootstock stem lesion-associated virus (GRSLaV), lettuce chlorosis virus (LCV), lettuce infectious yellows virus (LIYV), little cherry virus 1 (LChV-1), little cherry virus 2 (LChV-2), mint virus 1 (MV-1), persimmon virus B (PeVB), pineapple mealybug wilt-associated virus 1 (PMWaV-1), plum bark necrosis stem pitting-associated virus (PBNSPaV), potato yellow vein virus (PYVV), raspberry leaf mottle virus (RLMV), rose leaf rosette-associated virus (RLRaV), strawberry chlorotic fleck-associated virus (SCFaV), strawberry pallidosis-associated virus (SpaV), sweet potato chlorotic stunt virus (SPCSV), tobacco virus 1 (TV1), tomato chlorosis virus (ToCV), tomato infectious chlorosis virus (TICV).

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*3.4. Recombination analysis*

Recombination events supported by at least six out of nine different algorithms in RDP4 [31] were considered as possible events (Table 3). Significant evidences of several potential recombination events were found (Table 3). For the US isolate, the recombinant region covered a part of the ORF 2 (1b protein) and stretched to the p6 ORF and was predicted to involve the BC28074 and GR lineages as major and minor parents, respectively. In contrast, the G55 isolate was estimated to arise as a recombination event between the US and GR lineages with a predicted recombinant area covering the nearly complete 1a/1b (Table 3).

**Table 3.** Recombination analysis of genomic sequences of BcLRaV-1 isolates

| Recombinant BcLRaV-1 isolate | Position of the recombinant part | Putative parental isolates[1] | | Detection method | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Major parent | Minor parent | RDP | GENECONV | Bootscan | Maxchi | Chimaera | SiScan | PhylPro | LARD | 3Seq |
| G55 | 45-9957 | US | GR | 1.1E-52 | NS[2] | 5.1E-40 | 2.2E-15 | 4.6E-33 | 2.3E-84 | NS[2] | NS[2] | 1.6E-05 |
| US | 7701-10345 | BC28074 | GR | 2.7E-05 | NS[2] | 2.3E-02 | 1.5E-04 | 7.5E-06 | 5.8E-04 | NS[2] | 4.3E-51 | 2.2E-05 |
| US | 4514-4729 | GR | G55 | 1.0E-03 | 2.5E-02 | 7.2E-03 | NS[2] | 4.3E-02 | 8.5E-09 | NS[2] | NS[2] | 4.1E-02 |

[1] Major and minor parents – predicted contribution of larger and smaller sequence fragment, respectively, [2] NS – no support detected.

Recombination is one of the mechanisms that facilitate viral evolution. For several closteroviruses and ampeloviruses recombination events suggested to have an ancient origin were shown [12, 31]. Rather unexpected was the prediction that isolates from black currant (BC28074/US) or red currant (GR) were involved in the recombination process, suggesting a complex evolutionary history of BcLRaV-1. The number of analyzed isolates was however too low to trace any diversity patterns and their possible relationships.

## 4. Conclusions

Several diverse isolates of a novel closterovirus were found and later confirmed in red and black currant with unclear disease phenotype in Europe and North America. Sequence analyses of whole genome and phylogenetic inference confirmed that they all belong to one novel viral species of the genus *Closterovirus*, family *Closteroviridae*, tentatively named 'blackcurrant leafroll associated virus 1' (BcLRaV-1). The presence of the virus was further confirmed with electron microscopy and sequencing of RT-PCR amplicons. Phylogenetic analyses of selected viral proteins revealed topological differences between the 1b and CP/HSP70h proteins-based trees that might evidence to potential intraspecies recombination events. Indeed, several possible recombination points with significant support were located (Table 3). It may indicate complex transmission routes that enabled co-infection of a single host by the hypothetical parental genomes in the past.

Genetically nearly identical US and SLO isolates (Fig. 3) may reflect a long-distance movement of virus-infected *Ribes* plants. Otherwise, the number of identified BcLRaV-1 isolates and their genetic divergence does not allow discrimination of any molecular or host-related groups. Further investigation should evaluate whether BcLRaV-1 causes disease.

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