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Cherry leaf roll virus – an Emerging Virus in Finland?

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Cherry leaf roll virus, CLRV, is a plant pathogen that infects a variety of deciduous trees and shrubs in temperate regions. Little is known about its occurrence at high latitudes and especially in Finnish birch species. Still, symptoms that seemed to be associated with CLRV such as vein banding, leaf roll and decline have been observed in birch trees throughout the country since the summer of 2002. Six different birch species, subspecies or varieties, i.e. *Betula pubescens* subsp. *pubescens* (downy birch), *B. pendula* (silver birch), *B. nana* (dwarf birch), *B. pubescens* var. *appressa* (Kiilopää birch), *B. pubescens* subsp. *czerepanovii* (mountain birch) and *B. pendula* var. *carelica* (curly birch) originating from all over Finland were assessed by immunocapture-reverse transcription-polymerase chain reaction (IC-RT-PCR) for CLRV infection. It was shown that CLRV is widely distributed in *B. pendula* and *B. pubescens* throughout the country. Furthermore, dwarf birch, mountain birch, Kiilopää birch and curly birch were confirmed to be previously unkown hosts of CLRV. Genetic analysis of virus sequence variants originating from Finnish birch trees revealed atypical phylogenetic relationships. In contrast to CLRV isolates from birches growing in the United Kingdom and Germany which clustered exclusively within group A, Finnish CLRV isolates belonged either to group B, D or E. Thus, virus population structure in Finnish birches seems to be more variable and host plant dependency seems not to apply for Finnish CLRV isolates.

Keywords *Betula nana, Betula pendula, Betula pubescens,* IC-RT-PCR, 3´ non-coding region, CLRV, phylogenetic relationship
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1 Introduction

A severe and increasing birch decline in different parts of Finland is observed since 2002. Trees suffer from loss of vigour, and leaves indicate vein banding, leaf roll, chlorosis and subsequent necrosis. Referring to these symptoms they lead to the assumption that they are caused by viruses. In a survey covering major parts of the country, symptoms on birch were especially distinct after the extremely dry summer of 2006, and several birch species are affected. Trees showed CLRV-characteristic symptoms and recently, this virus was confirmed in Rovaniemi, northern Finland in several *Betula pubescens* Ehrh. subsp. *pubescens* (downy birch) trees (Jalkanen et al. 2007).

Cherry leaf roll virus (CLRV) belongs to the subgroup c of the genus *Nepovirus* (family *Comoviridae*). The two (+)ssRNAs of the bipartite viral genome each contain an extraordinary long 3´ non-coding region (3´ NCR, Borja et al. 1995, Scott et al. 1992), which is involved in regulation of replication and translation (Dreher 1999) as described for *Blackcurrant reversion nepovirus* (Karetnikov et al. 2006). Comparative studies of CLRV strains from different origins done by Rebenstorf et al. (2006) revealed that the sequence variability of a short stretch of the 3´ NCR near the 3´ terminus of the virus RNA leads to grouping of virus isolates into different phylogenetic clades. This clustering can be correlated with serological and biological properties of virus strains. Additionally, it was demonstrated that variability within this part of the virus genome is linked to the originating host plant species of the virus isolates. CLRV infects various deciduous trees and shrubs (Bandte and Büttner 2001) of temperate regions. It is a seed and pollen-borne virus, which is also transmitted by mechanical means, grafting, root connation and was recently reported to be water transmissible as well (Bandte et al. 2007).

More than one fifth of the Finnish forests are dominated by birch species. In total, the genus *Betula* represents the most common group of deciduous trees, which comprises an important raw material in the mechanical and chemical forest industry (Peltola 2007). The species *Betula pubescens* subsp. *pubescens* (downy birch) and *B. pendula* Roth (silver birch) are preferably used industrially for plywood, veneer (Luostarinen and Verkasalo 2000) and paper production (Viherä-Aarnio and Velling 1999). Downy and silver birches are early-succession tree species abundant throughout the country in towns, on roadsides and most common in mixed forests. However, north of the Arctic Circle also *B. nana* L. (dwarf birch), *B. pubescens* var. *appressa* (Kallio & Y. Mäkinen, Kiilopää birch) and *B. pubescens* subsp. *czerepanovii* (Orlova) Hämet-Ahti (mountain birch) are the specialised *Betula* species, comprising important key components of the sub-arctic ecosystem (Walker 2000, van Wijk et al. 2005). The decorative wood of curly birch (*B. pendula* var. *carelica* (Mercklin) Hämet-Ahti), which itself is an ornamental tree, is of great economic value in Finland providing the highest prized timber used in furniture and craftwork (Velling et al. 2000).

The aim of the study was to investigate the occurrence of CLRV in birch species and the distribution of the virus in Finland. Furthermore, it was intended to give a first assessment of characteristics of individual CLRV sequence variants obtained from different locations in Finland and *Betula* species. This includes the comparison of the CLRV strains found in Finland with other known virus isolates which have already been phylogenetically characterised by Rebenstorf et al. (2006).

2 Material and Methods

Seventy-seven trees exhibiting characteristic symptoms of a virus infection (34 *B. pubescens* subsp. *pubescens*, 27 *B. pendula*, six *B. pubescens* subsp. *czerepanovii*, five *B. pubescens* var. *appressa,* four *B. nana* and a single *B. pendula* var. *carelica*) were sampled all over Finland. Two symptomatic twigs including catkins and young leaf tissue of individual trees were collected between the height of 2–5 m of trees which were between 5 and 100+ years old. Twigs were wrapped in moist paper in a polyethylene bag and cooled. The samples were sent within one week to the laboratory and were processed immediately or kept frozen at –20 °C until molecular analysis. Sampled trees were tested in duplicate by appli-

cation of homogenates of symptomatic leaves and buds, catkins or twig tips in a CLRV specific immunocapture-reverse transcription-polymerase chain reaction (IC-RT-PCR) described in Jalkanen et al. (2007) in detail. CLRV particles were coated to the wall of reaction tubes by a virus specific polyclonal antibody (10 µl of a 1:200 dilution of 1 mg/ml IgG) which was developed in rabbit against an elderberry isolate of CLRV (Gentkow et al. 2007). The virus RNA becomes accessible after washing away plant material and is copied into a cDNA by 200 units of the enzyme M-MuLV reverse transcriptase (Fermentas, Germany). The generated cDNA is then amplified by polymerase chain reaction applying 2 units GoTaq flexi DNA polymerase (Promega, Wisconsin, USA) with the supplied buffer and a final concentration of 2 mM $MgCl_2$. Specific oligonucleotides developed by Werner et al. (1997) were used in PCR targeting the partial 3´ NCR of CLRV which have been shown suitable to investigate the phylogenetic relationships of virus isolates within the species (Rebenstorf et al. 2006). PCR was conducted in a Tgradient thermocycler (Biometra, Germany) with the following program: 4 min initial denaturation at 94 °C followed by 30 cycles of denaturation for 1 min at 94 °C, annealing 45 s at 55 °C and elongation for 1 min at 72 °C; final extension was for 5 min at 72 °C. Amplification products of the IC-RT-PCR were separated by electrophoresis in 1% (w/v)-agarose gels. Nucleic acids were stained by incubation of gels in ethidiumbromide (1 µg/ml TBE-buffer) and visualised under uv-light (312 nm). A sample tree was scored as CLRV infected, if a specific fragment of the expected size (404–420 bp) was detected in at least one sample per tree.

Selected IC-RT-PCR generated fragments of the CLRV 3´ non-coding region that were obtained from three downy birches of Rovaniemi, two silver birches and one mountain birch from different locations in Finland (Lieksa, Vaasa, Inari) were analysed by sequencing based on the chain-termination method developed by Sanger et al. (1977). Amplified fragments were ligated into pBluescriptII SK(-)-vectors (Stratagene, La Jolla, USA) and transformed into *E. coli* (JM109, Promega, Wisconsin, USA) using standard protocols (Sambrook et al. 1989). Plasmid-DNA was purified with the Invisorb Spin Plasmid Mini Kit (Invitek, Berlin, Germany) according to manufacturer's instructions. Cloned inserts were sequenced from both directions using a BigDye Terminator v1.1 Ready Reaction Cycle Sequencing Kit (Applied Biosystems, Foster City, USA) after standard protocol with vector specific primers and an ABI PRISM 310 Genetic Analyzer (Applied Biosystems, Foster City, USA). Obtained sequences were assembled in the sequence alignment editor software BioEdit 7.0.5.2 (Hall 1999). They were subjected to an online database search using BLAST (Basic Local Alignment Search Tool, Altschul et al. 1997) at the National Center for Biotechnology Information (NCBI) to retrieve related sequences with significant scores. CLRV sequences obtained from Finnish birches were aligned and compared with corresponding nucleotide fragments extracted from the NCBI database (Table 1) applying the computer program ClustalX 1.83 (Thompson et al. 1997). After removal of primer sequences pairwise sequence identities were calculated from resulting sequence fragments. Comparison of the pairwise sequence identities of the group of CLRV sequence variants originating from Finland with diversities of a second group of birches from other geographic origins were done by calculation of mean values and standard deviation of the two groups. Phylogenetic trees were inferred from the sequence alignment using neighbour-joining, maximum-likelihood, and maximum-parsimony methods incorporated in the BioEdit software package in order to apply different algorithms. To test for statistical robustness of data, the neighbour-joining tree was calculated with ClustalX 1.83 including bootstrap analyses with 1000 repetitions; other parameters were left in default settings.

3 Results

The sampled trees from southern, central and northern Finland revealed numerous CLRV infections. Results confirm that CLRV is widely distributed throughout Finland (Fig. 1), even north of Rovaniemi and the Arctic Circle up to the northern and alpine tree line. Tree samples originated from rural areas, i.e. from alleys, parks (churchyard, schoolyard) and along roadsides in

Table 1. CLRV isolates from the database included in the phylogenetic analyses.

Accession no. [a)]	CLRV isolate	Host plant	Geographic origin	Phylogenetic group [b)]
AB168098	chinese chive	*Allium tuberosum* Rottl. ex Spreng.	Japan	C
AB168099	Rumex AGBC	*Rumex acetosella* L.	Japan	C
AB168100	Rumex acetosella-21	*Rumex acetosella* L.	Japan	C
AJ877118	E120	*Betula pendula* Roth	Berlin, Germany	A
AJ877119	E499s	*Betula pendula* Roth	Berlin, Germany	A
AJ877120	E896s	*Betula pendula* Roth	Berlin, Germany	A
AJ877121	E696s	*Betula pendula* Roth	Hamburg, Germany	A
AJ877122	E111	*Betula pendula* Roth	Hamburg, Germany	A
AJ877123	E806	*Betula pendula* Roth	United Kingdom	A
AJ877124	E1469	*Betula pendula* Roth	United Kingdom	A
AJ877125	E836s	*Betula nigra* L.	Lower Saxony, Germany	A
AJ877126	GAY	*Juglans regia* L.	United Kingdom	D1
AJ877127	E327	*Prunus avium* L.	North-Rhine-Westfalia, Germany	A
AJ877128	E803	*Prunus avium* L.	United Kingdom	A
AJ877129	E1472	*Prunus avium* L.	United Kingdom	A
AJ877130	E676s	*Sambucus nigra* L.	Schleswig-Holstein, Germany	B
AJ877131	E485	*Sambucus nigra* L.	Mecklenburg-Western Pomerania, Germany	E
AJ877132	E603	*Sambucus nigra* L.	Brandenburg, Germany	E
AJ877133	E583	*Sambucus nigra* L.	Brandenburg, Germany	E
AJ877134	E119s	*Sambucus nigra* L.	Berlin, Germany	E
AJ877135	E622s	*Sambucus nigra* L.	Berlin, Germany	E
AJ877136	E839s	*Sambucus nigra* L.	Berlin, Germany	E
AJ877137	E541s	*Sambucus nigra* L.	Berlin, Germany	E
AJ877138	E443	*Sambucus nigra* L.	Berlin, Germany	E
AJ877139	E441	*Sambucus nigra* L.	Saxony-Anhalt, Germany	A
AJ877140	E950s	*Sambucus nigra* L.	Saxony-Anhalt, Germany	E
AJ877141	E568	*Sambucus nigra* L.	North-Rhine-Westfalia, Germany	E
AJ877142	E576	*Sambucus nigra* L.	North-Rhine-Westfalia, Germany	E
AJ877143	E492	*Sambucus nigra* L.	Hungary	E
AJ877144	PV-0276	*Sambucus nigra* L.	North-Rhine-Westfalia, Germany	E
AJ877145	E804	*Sambucus canadensis* L.	United States of America	E
AJ877146	E326	*Juglans regia* L.	North-Rhine-Westfalia, Germany	D1
AJ877147	E648	*Juglans regia* L.	France	D1
AJ877148	4WJUG	*Juglans regia* L.	United Kingdom	D1
AJ877149	E800	*Juglans regia* L.	United Kingdom	D1
AJ877150	E156	*Juglans regia* L.	Hungary	D2
AJ877151	CTIFL	*Juglans regia* L.	France	D1
AJ877152	Ludmila	*Juglans regia* L.	Slovakia	D2
AJ877153	E697s	*Sorbus aucuparia* L.	Hamburg, Germany	B
AJ877154	E695s	*Sorbus aucuparia* L.	Schleswig-Holstein, Germany	B
AJ877155	E693	*Sorbus aucuparia* L.	Baden-Württemberg, Germany	E
AJ877156	E141s	*Carpinus betulus* L.	North-Rhine Westfalia, Germany	E
AJ877157	E575s	*Aegopodium podagraria* L.	North-Rhine Westfalia, Germany	B
AJ877159	E113	*Fagus sylvatica* L.	North-Rhine Westfalia, Germany	A
AJ877160	E801	*Ulmus americana* L.	United States of America	A
AJ877161	E797	*Cornus florida* L.	United States of America	A
AJ877162	E802	*Rubus idaeus* L.	New Zealand	C
AJ877163	E805	*Rubus fruticosus* L.	United Kingdom	A
AJ877164	E1636	*Vitis vinifera* L.	Rhineland-Palatinate, Germany	A
AJ877165	E395	*Rheum rhabarbarum* L.	North-Rhine Westfalia, Germany	B
AJ888533	E678s	*Fraxinus excelsior* L.	Bavaria, Germany	B

Accession no. [a]	CLRV isolate	Host plant	Geographic origin	Phylogenetic group [b]
AJ888534	E698s	*Fraxinus excelsior* L.	Rhineland-Palatinate, Germany	B
EF182751	Edremit 2	*Juglans regia* L.	Turkey	-
S84124	I2-RNA1	*Betula pendula* Roth	United Kingdom	A
S84125	I2-RNA2	*Betula pendula* Roth	United Kingdom	A
S84126	R25	*Rheum rhabarbarum* L.	United Kingdom	B
U24694	W8-RNA2	*Juglans regia* L.	United States of America	D1
X99828	PetHH1	*Petunia hybrida*	Hamburg, Germany	-
Z344265	W8-RNA1	*Juglans regia* L.	United States of America	D1

[a] Database accession via National Center for Biotechnology Information (NCBI)
[b] According to Rebenstorf et al. 2006

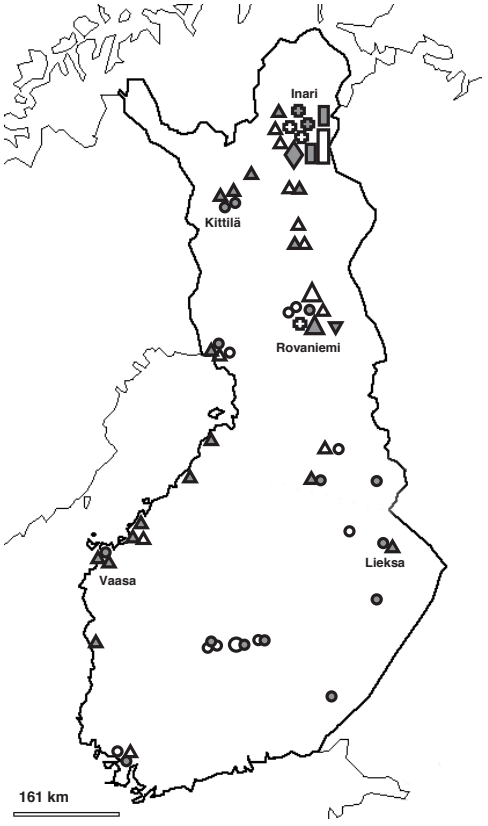

Fig. 1. Location of sampled trees expressing virus-like symptoms in Finland. Species are indicated by the following symbols: *Betula pubescens* subsp. *pubescens* (△), *B. pendula* (○), *B. pubescens* subsp. *czerepanovii* (□), *B. pubescens* var. *appressa* (◇), *B. nana* (+), and *B. pendula* var. *carelica* (▽). CLRV infected trees confirmed by IC-RT-PCR are indicated by dark symbols. Small symbols represent one individual tree, large symbols 4–5 trees.

Table 2. Detection of CLRV by IC-RT-PCR in symptomatic birch species in Finland.

Species	Sampled, no.	CLRV positive, no.
B. pubescens subsp. *pubescens*	34	20
B. pendula	27	14
B. pubescens subsp. *czerepanovii*	6	2
B. pubescens var. *appressa*	5	5
B. nana	4	2
B. pendula var. *carelica*	1	1
Total	77	44

town centres but also from natural stands as for instance the samples collected in Inari. Altogether, CLRV infection was proved in 44 out of 77 of the symptomatic trees, thereof 20 downy birches and 14 silver birches (Table 2).

As an example, CLRV could be detected in all birch species growing in the municipality of Inari (Lapland); the number of the sampled dwarf birches (4), mountain birches (6) and Kiilopää birches (5) included in the assay was limited; still, CLRV detection was successful at least in two trees per species. Additionally, the one curly birch sampled from a garden in Rovaniemi was CLRV positive. This is the first time that these species have been confirmed to be hosts for the virus. Additionally, four CLRV infected saplings – two *B. pendula* and two *B. pubescens* – originated from a 100-year-old seed-production stand in Sätkenä, Kittilä. So far CLRV has never been recorded north of the Arctic Circle or in *B. pubescens* and *B. pendula* trees in the entire country.

Table 3. Characteristics of CLRV isolates obtained from various Finnish birch trees.

Accession no.	Species, tree no., tissue	Origin, sampling date	CLRV isolate, clone	Fragment length [a], bp	Phylogenetic group [b]
AM981029	*B. pubescens* subsp. *pubescens*, 1, leaf	Rovaniemi, May 2007	E2484, EG1	412	B
AM981030	*B. pubescens* subsp. *pubescens*, 3, leaf	Rovaniemi, May 2007	E2485, EG3	412	E
AM981031			E2485, EG12	412	B
AM981032	*B. pubescens* subsp. *pubescens*, 20, leaf	Rovaniemi, July 2007	E2501, EG9	412	E
AM981033			E2501, EG10	412	E
AM981034	*B. pendula*, 35, leaf	Lieksa, July 2007	E2532, EG22	404	D1
AM981035	*B. pendula*, 55, twig tip	Vaasa, July 2007	E2558, EG28	412	E
AM981036	*B. pubescens* subsp. *czerepanovii*, 98, bud	Inari, July 2007	E2621, EG31	404	D1
AM981037			E2621, EG32	412	B

[a] IC-RT-PCR product including primer sequences
[b] According to Rebenstorf et al. (2006)

CLRV infection of three downy, two silver birches and one mountain birch was confirmed by sequencing (Table 3). CLRV sequence fragments of the 3´NCR received from Finnish birches have been submitted to the sequence database maintained by the European Molecular Biology Laboratory (EMBL) and are available by the accession numbers AM981029–AM981037. These are the first partial sequences available for CLRV obtained from Finnish host plants. The samples originated from various locations in Finland, i.e. Rovaniemi and Inari (North), Lieksa (East) and Vaasa (West) (Fig. 1), and display size differences of the partial 3´ NCR between 404 and 412 bp (Fig. 2) as well as sequence variability. Phylogenetic analyses of CLRV sequences applying maximum-likelihood or maximum parsimony algorithms led to similar grouping and confirmed the neighbour-joining tree (Fig. 3). Sequence fragments of CLRV obtained from Finnish birches shared highest sequence identities to CLRV isolates characterised previously belonging to phylogenetic group B, D1 or E. Two identical sequences derived from different sample trees (EG22 and EG31) clustering within phylogenetic group D1 were 363 bp long excluding primer sequences. This is due to a deletion of eight consecutive nucleotides at position 132–140 within the alignment (data not shown). Interestingly, this deletion is also present in other CLRV isolates previously characterised as members of group D1 (accessions AJ877126, AJ877146, AJ877147, AJ877148, AJ877149, AJ877151, U24694, Z34265). Seven of the inves-

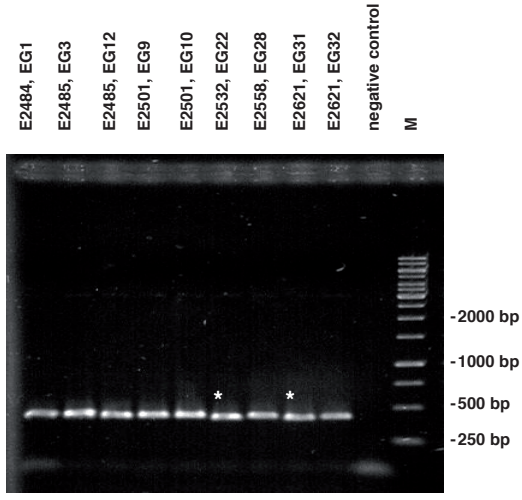

Fig. 2. CLRV specific fragments of the partial 3´ non-coding region (412 bp) amplified by IC-RT-PCR from different birch trees. Sample names are according to Table 3. As the negative control is water instead of nucleic acid template applied in PCR. The marker M is the 1 kb Ladder, Fermentas, Germany and sizes in basepairs (bp) of some reference fragments are indicated on the right. The asterisks indicate the shorter fragments of CLRV sequence variants of 404 bp.

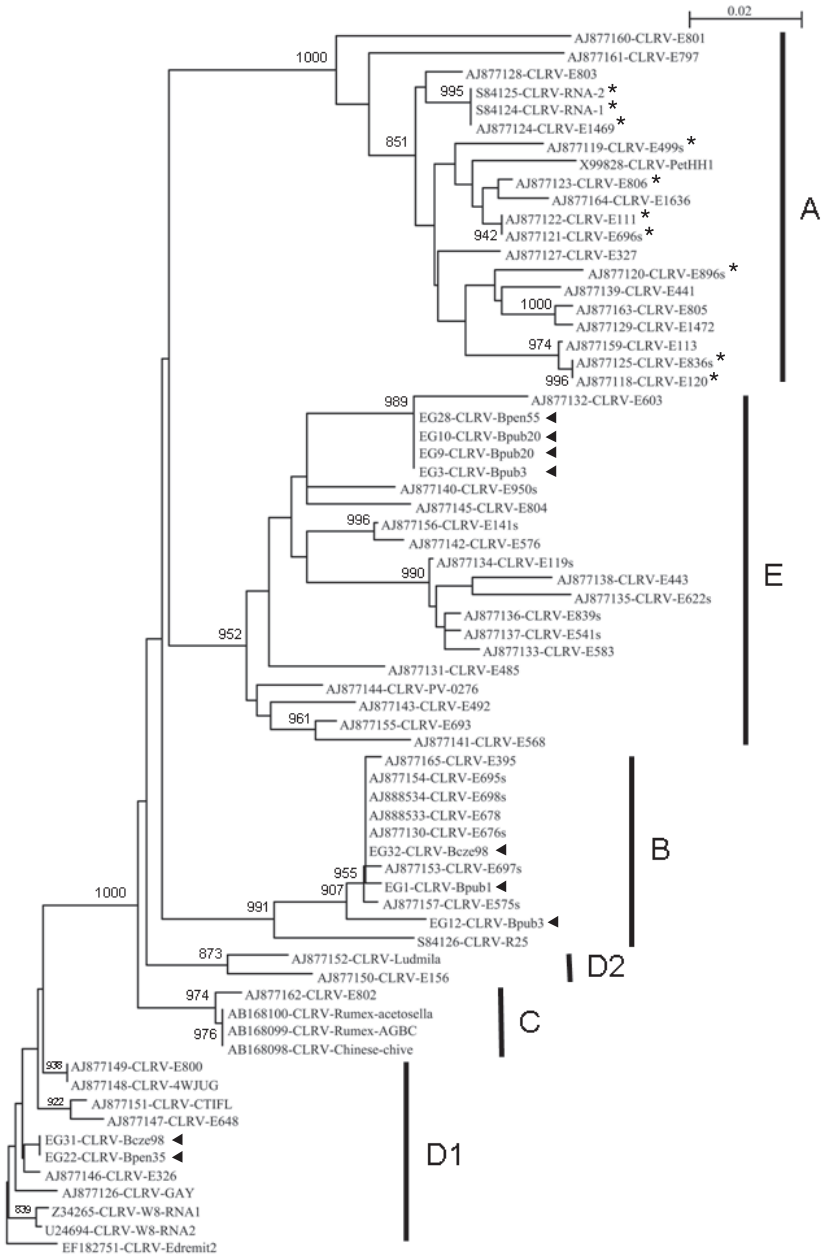

Fig. 3. Phylogenetic tree inferred from the nucleotide sequence alignment (386 bp) of the 3´ non-coding regions of *Cherry leaf roll virus* isolates generated with ClustalX 1.83 applying the neighbour-joining algorithm. Bootstrap analysis was performed with 1000 repetitions and values above 800 are indicated at branch nodes. The scale bar of 0.02 represents 2 nucleotide substitutions per 100 nucleotides of the aligned sequences. Sequences obtained from the NCBI database are indicated by their accession numbers according to Table 1. CLRV sequences obtained from *Betula* spp. in Finland (Table 3) are indicated with arrowheads, isolates originating from *Betula* spp. in Germany and United Kingdom are pinpointed with asterisks. Phylogenetic groups (A to E) according to Rebenstorf et al. (2006) are indicated by bold lines on the right.

Table 4. Sequence identities of CLRV sequences after pairwise comparison received from the *Betula* spp. group from Finland and the group from United Kingdom or Germany.

Parameter	n [a)]	Mean (S.D.)	Min.	Max.
Subgroup sequence identity				
CLRV, United Kingdom, Germany [c)]	10	0.968 (± 0.016)	0.930	1 [b)]
CLRV, Finland [d)]	9	0.930 (± 0.042)	0.892	1
Sequence identity after pairwise comparison of sequences between subgroups				
CLRV from *Betula* spp.	19	0.875 (± 0.012)	0.848	0.893

[a)] Sequences included in the pairwise comparison
[b)] Identical sequences
[c)] Isolates originating from *Betula* spp. in the United Kingdom and Germany are indicated in Table 1.
[d)] See Table 3

tigated sequences were 371 bp in length excluding primer sequences. Four clones (EG3, EG9, EG10 and EG28) revealed identical sequences and showed highest sequence identities to CLRV isolates determined as phylogenetic group E (Fig. 3); three Finnish CLRV sequence variants (clones, EG1, EG12 and EG32) related to phylogenetic group B isolates and showed sequence variability of max. 2.2%. It was found that a single birch tree harbour different CLRV sequence variants. In detail, one individual clone from *B. pubescens* subsp. *pubescens* (CLRV-E2485, EG3) relates to group E, while the second (CLRV-E2485, EG12) represents group B; sequences differed in 30 positions of 371 aligned nucleotides (identity score of 0.919). Two different CLRV clones sequenced from *B. pubescens* subsp. *czerepanovii* belonged either to group B (CLRV-E2621, EG32) or D1 (CLRV-E2621, EG31) exhibiting a slightly lower sequence identity of 0.913. A higher variability of sequence fragments obtained from CLRV infected birches in Finland was substantiated by comparison of the group of nine Finnish sequence variants (Table 3) with a subgroup of ten CLRV isolates originating from *Betula* sp. in the United Kingdom and Germany available in the database (accessions AJ877118, AJ877119, AJ877120, AJ877121, AJ877122, AJ877123, AJ877124, AJ877125, S84124, S84125). The group of Finnish CLRV strains revealed a lower mean intra-group sequence identity of 0.93 within the analysed region of the 3´ NCR (Table 4). Lowest identity scores of 0.892 were determined for the Finnish sequence variants relating to phylogenetic group D1 (clones EG31 and EG22) and E (EG3, EG9, EG10 and EG28). Birch CLRV isolates from different locations in the UK and Germany, all clustering within phylogenetic clade A, show at least mean identities of 0.968 with a minimum sequence identity of 0.930. Mean inter-group sequence identities of these two CLRV groups originating either from Finland or from other European countries was even lower and ranged between 0.848–0.893.

4 Discussion

CLRV has been confirmed in symptomatic birch trees from several places in Finland by molecular means, revealing that the virus is widely distributed in the country and also affects at least six birch species native to Fennoscandia. The main route of CLRV dispersal in birch in natural habitats is assumed to be pollen and seed transmission, which has been studied in detail before (Cooper 1976, 1979, Cooper et al. 1984). Hybridisation and cytoplasmic introgression occur frequently within the genus *Betula* and hybrids of silver, downy, and dwarf birch are reported from Scandinavia as well (Thórsson et al. 2001, Palme et al. 2004). Thus, interspecific fertilisation by CLRV infected pollen may contribute to virus dispersal within this genus. However, pollen mediated vertical transmission as an exclusive mode of CLRV dissemination cannot sufficiently explain the sudden appearance of symptoms in CLRV

infected birch trees or the wide distribution all over the country. As birch pollen is anemophilous, one would expect virus-induced symptoms first on wind exposed sites and sides of the canopy. This seems not to be the case, because sampled trees from sheltered sites within forest stands were also found to be affected by the disease. Vertical transmission by seed may also be hampered by lower germination rates and less viable CLRV infected birch seedlings (Cooper et al. 1984). Still, in our investigations we found CLRV infected silver and pubescent seedlings in a seed production stand in Kittilä in northern Finland. Thus, contaminated seed could be a possible route of CLRV dispersal into planted birch populations, which exist for instance as alleys in Finnish town centres. However, most alleys and the symptomatic town birches are rather old, 40 to 80 years, suggesting that contaminated seed is not involved in the rapid spread of CLRV unless the trees are infected without symptoms.

Sequence comparisons revealed an unusual phylogenetic relationship of Finnish CLRV isolates. Until now, phylogenetically characterised CLRV isolates of birch trees from the United Kingdom and Germany exclusively cluster within clade A (Rebenstorf et al. 2006). It was shown that CLRV isolates could be genetically differentiated according to the originating host plant species. From this finding the authors concluded that co-evolution of CLRV with the host plant species is an important factor responsible for the quick genetic adaptation of the virus population to the host species. In our study we found that CLRV sequence variants from Finnish birch species did not cluster in group A with previously characterised CLRV isolates originating from birch. For instance two Finnish CLRV sequence variants from silver birch and mountain birch (CLRV-E2532, EG22, CLRV-E2621, EG31) clustered within phylogenetic group D. This is remarkable, because in this clade CLRV isolates originating only from walnut trees were established to date and therefore the co-evolutional mechanisms postulated by Rebenstorf et al. (2006) seem not to apply to CLRV found in Finnish birches. Instead our findings suggest that a more diverse CLRV population is present in Finnish birches, which may also explain the wide occurrence and the aggressive spreading of the virus in Finland in a very short time. This may be due to new introduction of CLRV into the region. However, this cannot be secured because of the few individuals characterised so far. The majority of nine sequenced clones was found to relate to group B or represent E variants. Both phylogenetic clades comprise CLRV isolates of a wider range of host plant species including *Fraxinus excelsior* L., *Sorbus aucuparia* L. and *Sambucus* sp. (Rebenstorf et al. 2006), which are native to southern Finnish ecosystems, too (Mikk and Mander 1995, Simola 2006). This may indicate that CLRV strains of these phylogenetic groups are not restricted in host range and are able to infect various plant genera. Thus, CLRV infections in Finland are most likely not restricted to the genus *Betula*, but the virus may also infect other trees and shrubs of the Finnish flora.

On the contrary, birches may have recently acquired CLRV from other host plants, and the virus population is not adapted to specific host plant species yet. This is substantiated by a single report from Cooper and Edwards (1980), who mentioned a CLRV isolate that was obtained in Finland from *Sambucus racemosa* L. This would imply other routes of CLRV transmission than by pollen or seed to be important in Finland. Interspecific viral spread might be facilitated by mechanical inoculation, either with the assistance of suitable insect vectors (Werner et al. 1997) or through virus entrance into roots via CLRV contaminated soil and water in conjunction with wounding. As it is still unknown, whether insects, mites or nematodes are involved in the transmission of this virus, the contributing agents of CLRV dissemination in the Finnish environment have to be investigated in the future. In particular, global warming may lead to a changed ecosystem, which alters the plants physiological condition. According to Garret et al. (2006) this may contribute to higher susceptibility of plants causing increased symptom development after virus infection or enhancing virus replication capabilities within the host plant. Climate change predictions for Finland conducted by Jylhä et al. (2004) estimate that annual mean temperatures as well as precipitation will raise within the next fifteen years, including significantly affected seasonal temperatures. Plant species sensitive to elevated temperatures may respond to a warmer climate with loss of virus tolerance, thus facilitating virus replication in the host plant.

Also water transmission of CLRV has to be taken into serious consideration (Bandte et al. 2007). Earlier thawing of ice and ground frost in combination with an earlier start of the vegetation period may stress plants to loose their resistance to pathogens. Although the response to warming is generally understood, concurrent changes in other climatic factors may affect plant–pathogen interactions, leading to a shift in disease distribution as outlined by Garret et al. (2006).

CLRV infection may affect the local wood industry because of its abundance in the economically important birch species *B. pubescens* and *B. pendula.* The virus influence on machining quality is not yet known, but its appearance in forest trees may favour other predisposing and inciting environmental factors of decline, which may entail reduced tree vitality as well as wood quality. Referring to the rate at which symptoms have spread within the last few years, CLRV might become a serious problem in northern birch forest ecosystems. In combination with the expected climatic changes the virus may contribute to impair key species of the delicate sub-arctic plant communities like *Betula nana*. In order to prevent further distribution of CLRV, its early detection in birch trees as well as investigation of other possible host plants of the Finnish flora is necessary.

Finally, the etiology of the disease affecting at least six Finnish birch species has to be thoroughly investigated in the future. We could detect CLRV in only 57% of the symptomatic birch trees which may be explained by the applied method for virus detection including an immunocapture step. The polyclonal antibody which was developed against an elderberry isolate of CLRV may not recognise all strains of the virus, because it is known that the species consists of different serotypes (Rowhani and Mircetich 1988, Jones et al. 1990, Rebenstorf et al. 2006). Furthermore, it cannot be excluded that other viruses are involved in the disease affecting birches (Nienhaus and Castello 1989). Although the symptoms in birch are characteristic for an infection with CLRV (Schmelzer 1972, Cooper and Atkinson 1975), Koch´s postulates remain to be fulfilled in order to prove CLRV as causal agent of the disease observed in birch species in Finland.

Concluding, detailed studies are needed, especially concerning mode of CLRV transmission within birch species as well as virus dispersal within the Finnish environment including other possible hosts, because CLRV has emerged in a few years time in all Finnish regions and occurs in all birch species investigated to date. Additionally, ecological and economic impact of the emerging virus disease has to be estimated in the near future. It is of particular interest to determine the variability of CLRV sequences and monitor the development of CLRV virus populations found in Finnish plant species, because they differ considerably from previous findings and may enable the investigation of genetic adaptation processes of CLRV to different woody hosts.

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