Eur J Plant Pathol
DOI 10.1007/s10658-013-0246-z

Lichens—a new source or yet unknown host of herbaceous plant viruses?

Karel Petrzik · Jan Vondrák · Miloš Barták · Ondřej Peksa · Olga Kubešová

Accepted: 19 June 2013

Abstract Lichens are symbiotic associations of fungi with green algae or cyanobacteria. They have arisen independently several times within the *Ascomycota* and *Basidiomycota*. This symbiosis became with time one of the most successful life forms on Earth. Outside of the symbiotic algae and fungi, there are endophytic fungi, other algae, and lichen-associated bacteria present within lichen thalli. Till now, no lichen-specific pathogens have been reported among bacteria and viruses. Around 15 dsRNA viruses are known from *Eurotiomycetes* and another dsRNA and reverse transcribed ssRNA viruses from *Dothideomycetes* containing some lichenized fungal lineages. Algal viruses have been identified from less than 1 % of known eukaryotic algal species but no virus has been found in *Trebouxia* or in *Trentepohlia* (Chlorophyta, Pleurastrophyceae, Pleurastrales), the most common green lichen photobionts. On the other hand, dsDNA viruses infecting related *Chlorella* algae are well known from freshwater phytoplankton. However, high-molecular weight dsRNA isolated from different lichen thalli indicated to us presence of ss or dsRNA viruses. A PCR-based search for viruses with genus-specific and species-specific primers resulted in amplification of genome segments highly identical with those of plant cytorhabdoviruses and with Apple mosaic virus (ApMV). The nucleotide sequence of the putative lichen cytorhabdovirus showed high identity (98 %) with Ivy latent cytorhabdovirus. The nucleotide sequences of six Apple mosaic virus isolates from lichens showed high similarity with ApMV isolates from apple and pear hosts. The lichen ApMV isolates were mechanically transmitted to an herbaceous host and detected positive in ELISA 14 days thereafter, which support its infectivity on plants. We prepared axenic cultures of photobionts identified as *Trebouxia* sp. from this ApMV-positive lichen samples. All these cultures were positive for ApMV in RT-PCR test. We suggest that lichens as a whole (or their photobionts, more specifically) could serve as reservoirs for viruses, despite the fact that the way of transmission between different organisms is not

K. Petrzik (✉) · O. Kubešová
Department of Plant Virology, Institute of Plant Molecular Biology, Biology Centre of the Academy of Sciences of the Czech Republic,
Branišovská 31,
370 05 České Budějovice, Czech Republic
e-mail: petrzik@umbr.cas.cz

J. Vondrák
Department of Taxonomy, Institute of Botany, Academy of Sciences of the Czech Republic,
Zámek 1,
252 43 Průhonice, Czech Republic

M. Barták
Section of Plant Physiology, Department of Experimental Biology, Masaryk University,
Kamenice 5/A19,
625 00 Brno, Czech Republic

O. Peksa
The West Bohemian Museum in Pilsen,
Kopeckého sady 2,
30100 Plzeň, Czech Republic

Published online: 02 July 2013

clear. We showed that lichens could harbour several viruses simultaneously, as the plant cytorhabdovirus and ApMV were detected in the same host, also.

Keywords Rhabdovirus · Ilarvirus · ApMV · Lichenized fungi · Lichenized algae · *Trebouxia*

Introduction

Lichens are highly specialized fungi containing algal or cyanobacterial colonies in their thalli as a source of carbohydrates (Schwendener 1868). It is well known that lichens do not constitute a homogeneous group (Lutzoni et al. 2001; Hibbett et al. 2007) and have arisen independently on several occasions within the *Ascomycota* and *Basidiomycota* (Lutzoni et al. 2001). This symbiosis is one of the most successful life forms and wide-spread associations in nature (Beckett et al. 2008). Lichens are found in almost all terrestrial habitats from the tropics to the polar regions, and algae and fungi have expanded by means of this association into habitats with low nutrient supply, extreme temperatures, extreme light exposure, and low water availability where separately they would be rare or non-existent. Worldwide lichen biodiversity is estimated to include as many as 17,000 species (Nash 2008; classification based on the fungal partner).

The lichen thallus does not usually contain the fungal and algal partners exclusively. Organisms occurring frequently within lichen thalli are lichenicolous fungi (Clauzade et al. 1989), endophytic fungi (Li et al. 2007), other algae, and lichen-associated free-living bacteria (Cardinale et al. 2008; Bates et al. 2011). Although numerous organisms may be present inside lichen thalli, no lichen-specific pathogens have been reported among bacteria and viruses.

The general presence of viruses in flowering plants (Magnoliophyta) seems to be relatively common, as indicated by metagenomics data (Rosario et al. 2009; Roossinck et al. 2010; Al Rwahnih et al. 2011; Ng et al. 2011). There are slightly more than 1,000 virus species recognized to infect plants (King et al. 2012), although this number undoubtedly represents only a fraction of the true virus biodiversity in flowering plants.

The number of land plant species is estimated to be around 300,000. If algae present in oceans are to be included, that number increases by about 20,000 additional putative virus host species (Mora et al. 2011). Algae are also symbionts of many protozoa (about 70,000 species) and chromista (about 35,000 species). Although not all plant species must be hosts for specific viruses, we suggest a huge gap in the overall understanding of viral diversity, evolution, and ecology.

Since 1962, when mycoviruses were discovered in the white button mushroom (Hollings 1962), these have been identified in almost all fungal groups. High frequencies of virus infections in a number of fungal species found in field fungal isolates (2–65 %) suggest there to exist a great number of yet unrecognized mycoviruses (Ghabrial and Suzuki 2010). The majority of viruses known from fungi are known from *Leotiomycetes* and *Sordariomycetes*, but mycoviruses have been described also from *Dothideomycetes* and *Eurotiomycetes* containing some lichenized fungal lineages (Table 1; Lumbsch and Huhndorf 2009). No species-type or genome-type virus preference has been observed in fungi; while mycoviruses with dsDNA, ssDNA, dsRNA, or ssRNA genomes are known, those with the DNA genome are the rarest. Furthermore, several mycovirus types have been observed to be shared among fungal taxa, thus indicating that mycoviruses may be less specialized (Feldman et al. 2012). From this point of view, there is no reason to expect viruses to be absent in lichenicolous fungi. To date, however, there has been only one preliminary finding about viruses in the lichen *Cladonia fimbriata* (cup lichen). Two sequences obtained by deep-sequencing method have been identified as new chrysoviruses and one sequence as a new mitovirus (Narnaviridae) (http://bioinfosu.okstate.edu/pvbe/index.html).

Nearly 40 genera of algae and cyanobacteria have been reported as photobionts in lichen (Friedl and Büdel 2008). Algal viruses have been identified from less than 1 % of known eukaryotic algal species (Guiry and Rindi 2005), but no virus has been found in *Trebouxia* or in *Trentepohlia* (Chlorophyta, Pleurastrophyceae, Pleurastrales), the most common green lichen photobionts (Friedl and Büdel 2008) (Table 2.). On the other hand, dsDNA viruses infecting related *Chlorella* algae are well known from freshwater phytoplankton (Wilson et al. 2012).

Table 1 Viruses in fungal taxa close to lichen mycobionts[b]

	Acronym	Virus	Genome	Sequence/Genome size
Phylum ascomycota				
Class arthoniomycetes[a]		–		
Class dothideomycetes				
Order pleosporales[a]				
		Alternaria alternata REAL virus	reverse transcribed ssRNA	AB025309; 6 046 nt
	AaV-1	*Alternaria alternata*	4 linear dsRNA	NC_010989-91, NC_010984; 2 794 nt, 2 576 nt, 1 420 nt, 3 567 nt
	CmRV	*Coniothyrium minitans* RNA virus	dsRNA	NC_007523, 4 975 nt
	CThTV	*Curvularia* thermal tolerance virus	two linear dsRNA	NC_010985-6; 2 149 nt, 1 886 nt
	Hv145SV	*Helminthosporium victoriae* 145S virus	4 linear dsRNA	NC_005978-81; 3 612 nt, 3 134 nt, 2 972 nt, 2 763 nt
	Hv190SV	*Helminthosporium victoriae* 190S virus	single linear dsRNA	NC_003607; 5 179 nt
Order capnodiales	CfuTIV	*Cladosporium fulvum* CfT-1 virus	reverse transcribed ssRNA	Z11866; 7 396 nt
Order botryosphaericeae	DsRV1	*Diplodia scrobiculata* RNA virus 1	dsRNA	NC_013699; 5 018 nt
Class eurotiomycetes		about 15 viruses; with dsRNA genome from genera Chrysovirus, Partitivirus, Totivirus		
Class laboulbeniomycetes		–		
Class lecanoromycetes[a]		–		
Class leotiomycetes		more than 20 viruses; with ssRNA genome from genera Mitovirus, Endornavirus; with dsRNA genome from genera Hypoviruses, Partitiviruses, Totivirus, Victorivirus; one unassigned virus with ssDNA genome		
Class lichinomycetes[a]		–		
Class neolectomycetes		–		
Class orbiliomycetes		–		
Class pezizomycetes		about 5 viruses; with ssRNA genome from genus Mitovirus; with reverse transcribed RNA genome putative viruses from family Pseudoviridae and genus Metavirus		
Class pneumocystidomycetes		–		
Class saccharomycetes		about 10 viruses; with reverse transcribed RNA genome from family Pseudoviridae, and genera Hemivirus, and Metavirus; with dsRNA genome from genus Totivirus; with ssRNA genome from genus Narnavirus		
Class schizosaccharomycetes		2 viruses with reverse transcribed RNA genome from genus Metavirus		
Class sordariomycetes		more than 80 viruses; with ssRNA genome from genera Hypovirus, Mitavirus; with dsRNA genome from genera Chrysovirus, Mycoreovirus, Partitivirus, Totivirus, Victorivirus; with reverse transcribed RNA genome from genus Metavirus		
Class taphrinomycetes		–		
Class not assigned		about 15 viruses; with ssRNA genome from genus Mitovirus; with dsRNA genome from genus Victorivirus and from unassigned genus		

Table 1 (continued)

	Acronym	Virus	Genome	Sequence/Genome size
Phylum basidiomycota				
Class agaricomycetes				
Order agaricales[ac]	AbV-1	*Agaricus bisporus* virus 1	up to 5 linear dsRNA	X94361-2; 3 396 nt, 2 455 nt
	MBV	*Agaricus bisporus* (mushroom) bacilliform virus	single linear ssRNA	NC_001633; 4 009 nt
	MVX	*Agaricus bisporus* (mushroom) virus X	several? linear ssRNA	AJ421979-90
		Clitocybe odora virus 1	single linear ssRNA	NC_017003; 3 765 nt
	FvBV	*Flammulina velutipes* browning virus	two linear dsRNA	AB465308-9; 1 915 nt, 1 798 nt
		Lentinula edodes mycovirus HKB	single linear dsRNA	AB429556; 11 282 nt
		Pleurotus (Oyster mushroom) isometric virus II	single linear dsRNA	AY308801; 2 038 nt
	OMSV	*Pleurotus* (Oyster mushroom) spherical virus	single linear ssRNA	AY182001; 5 784 nt
	PeSV	*Pleurotus eryngii* spherical virus	single linear ssRNA	7800 nt
	PoV1	*Pleurotus ostreatus* virus 1	two linear dsRNA	NC_006960-1; 2 223 nt, 2 296 nt
	RhsV	*Rhizoctonia solani* virus 717	two linear dsRNA	NC_003801-2; 2 363nt, 2 206 nt
	RVM2	*Rhizoctonia* virus M2	several? linear dsRNA	U51331; 3 570 nt
		Tricholoma matsutake marY1 virus	RNA RT	AB028236; 6 875 nt
Order polyporales[ad]	PgV-TW2	*Phlebiopsis gigantea* mycovirus dsRNA 1	single linear dsRNA	NC_013999; 110 563 nt

[a] marked algae taxa containing lichen photobionts, Friedl and Büdel 2008

[b] Taxonomy based on Catalogue of Life: 21st December 2012

[c] With the exception of genera *Multiclavula* and *Omphalina* this order is exclusively nonlichen forming

[d] *Dictyonema* is the only lichen forming genus in this order

[e] only these viruses were mentioned, where nucleotide sequence was published

Table 2 Viruses in green algae (Chlorophyceae) close to lichen algal photobionts[a]

	Acronym	Virus	Genome	Sequence/Genome size
Class bryopsidophyceae				
Class charophyceae	CAV	*Chara australis* virus	single linear ssRNA	JF824737; 9 065 nt
Class chlorophyceae				
Order chaetopeltidales				
Order chaetophorales[b]				
Order chlorococcales[b]				
Order chlorocystidales				
Order microsporales				
Order oedogoniales				
Order phaeophilales				
Order prasiolales[b]				
Order tetrasporales				
Order ulotrichales				
Order volvocales	VcaLeuV	*Volvox carteri* Lueckenbuesser virus	reverse transcribed single ss RNA	U90320; 4 603 nt
	VcaOssV	*Volvox carteri* Osser Virus	reverse transcribed single ss RNA	X69552; 4 885 nt
Class klebsormidiophyceae				
Class mesostigmatophyceae				
Class pedinophyceae				
Class pleurastrophyceae				
Class trebouxiophyceae				
Order chlorellales[b]	PBCV-1	*Paramecium bursaria* chlorella NC64 virus	single dsDNA	NC_000852; 330 611 bp
	PBCV-FR483	*Paramecium bursaria* chlorella FR483 virus	single dsDNA	NC_008603; 321 240 bp
	ATCV-1	*Acanthocystis turfacea* chlorella virus 1	single dsDNA	NC_008724; 288 047 bp
Order microthamniales[b]		Lichen cytorhabdovirus	single linear (−)ssRNA	KC109143; partial sequence
	ApMV	Apple mosaic virus	3 linear ssRNA segments	KC469071; partial sequence
Order oocystales				
Order not assigned[b]				
Class ulvophyceae				
Order cladophorales				
Order codiolales				
Order dasycladales				
Order siphonocladales				
Order trentepohliales[b]				
Order ulotrichales				
Order ulvales				

[a] Taxonomy based on Catalogue of Life: 21st December 2012

[b] marked algae taxa containing lichen photobionts, Friedl and Büdel 2008

A presence of mycoviruses and/or algal viruses to some extent similar to presently known viruses could be expected in lichens. In our preliminary work, however, we unexpectedly detected rhabdovirus-like sequences in some fungal species (unpublished results). This was the first indication of a putative presence of herbaceous plant viruses also in lichens, and we have begun attempts to detect plant viruses in these organisms. In this paper, we present: a concise review of: (1) viruses found in fungi related to

lichenicolous fungi, and (2) viruses in green algae from the *Chlorella* cluster, and first proofs of virus presence in several lichen species.

Material and methods

Sampling Saxicolous (growing on rock), terricolous (growing on the ground), and corticolous (growing on bark) lichens were collected in the Czech Republic (50 samples), Norway (5 samples), Greece (5 samples), Alaska (5 samples), and Antarctica (6 samples).

Algae cultivation Photobionts were isolated from rehydrated thalli using the micro isolation centrifugal method of Gasulla et al. (2010) and cultivated on 1.5 % agar plates with 3 N (meaning threefold more nitrogen content in the form of $NaNO_3$) Bold's Basal Media (3 N-BBM) with a 12 h photoperiod at 20 °C for 30 days. Colonies were selected and subcultured onto Petri dishes containing 3 N-BBM medium supplemented with peptone (10 g/l) and glucose (20 g/l).

NA isolation Total nucleic acids (DNA or RNA) were isolated from 0.1 g fresh or rehydrated lichen thalli or from a pinhead amount of alga culture growing on agar plate using a DNA or RNA plant kit (Macherey-Nagel, Germany), respectively, according to the manufacturer's recommendations. The isolations included 15 min enzymatic treatment of the unwanted nucleic acid. The iScript™ cDNA synthesis kit (Bio-Rad, Hercules, CA, USA) was used for cDNA synthesis.

Amplification Virus screening was performed using cytorhabdovirus-specific primers DFf 5′-GAY TTY GAR AAR TGG AAY GG-3′ and LVr 5′-GAG IAC YTG RTT RTC ICC-3′ (Petrzik 2012) and with Apple mosaic virus (ApMV) – specific detection primers 108 N7 5′-TCG TGA AGA AGT TTA GGT TGG-3′ and Ap403 5′-CCA TCT CAC CCC TAC ATC GCA T-3′. The complete ApMV capsid protein (CP) gene was amplified with primers 87E5 5′-GGC CAT TAG CGA CGA TTA GTC-3′ and 87E6 5′-ATG CTT TAG TTT CCT CTC GG-3′, as published previously (Petrzik and Lenz 2002). The algal ITS region was amplified with ITS1 5′- CTG CGG AAG GAT CAT TGA TTC-3′ and ITS4 5′- TCC TCC GCT TAT TGA TAT GC −3′primers (White et al. 1990; Piercey-Normore and DePriest 2001). PCR products of expected size were gel-purified and sequenced with those primers used for amplification by the BigDye Terminator v3.1 Cycle Sequencing kit (Life Technologies).

Alignment and sequence analysis Nucleotide sequences and their *in silico*-transcribed amino acid sequences were compared using blastn and blastp with GenBank data. The phylogenetic tree was calculated using the maximum parsimony method and 1000 bootstrap replications in MEGA5 (Tamura et al. 2011).

Biological test For infectivity experiments of ApMV, lichen thalli were homogenized in 0.1 M phosphate buffer, pH 7.4, and used for mechanical inoculation of cucumber cotyledons and first true leaves of Flame nasturtium (*Tropaeolum* sp.), then cultivated in an insect-proof glasshouse. Symptoms were evaluated 14 days after inoculation.

Serological test Double antibody sandwich enzyme-linked immunosorbent assay was performed using a DAS-ELISA kit for ApMV (Bioreba AG, Reinach, Switzerland) according to the manufacturer's recommendations. About 0.1 g of dry lichen thallus or cucumber leaves were homogenized in Bioreba sample buffer, centrifuged briefly and analysed. Absorbance at 405 nm was read after 2 h.

Results

Rhabdoviruses

The cytorhabdovirus detection primers were designed inside the most conservative domain of the RNA polymerase gene sequence, which is present in all negative-sense viruses. Samples of *Usnea chaetophora* from Norway (LN2) and *Cladonia arbuscula* (LRoz1) from the Czech Republic were the only two of 50 tested samples (4 %) which produced a visible product of about 340 bp after 35 amplification cycles. The blastn alignment with the recently available rhabdovirus sequences showed high sequence identity (98 %) with Ivy latent virus 1 (IvLV1, GenBank accession number GQ249163) for both of our sequences. The two sequences from lichen differ at 6 nt positions and at two amino acid

positions, when translated. Phylogenetic analysis of the *in silico* translated sequences classified both sequences into one cluster with Ivy latent virus 1 isolates (Fig. 1). A nucleotide sequence identity value of 64 % has been regarded as sufficient proof of a new rhabdovirus identity (Tao et al. 2008). This is much less than that observed among the IvLV1 isolates and the sequences from lichens (85–98 %). Comparison of longer sequences will be necessary for definite determination as to the virus's identity and its phylogenetic position.

Apple mosaic virus

In ELISA pre-screening of collected samples, 8 samples exceeded by at least two-fold the OD_{405nm} for the negative control (Fig. 2). All these samples amplified product of expected size about 260 bp for ApMV in PCR with detection primers. The capsid protein gene of ApMV was sequenced from six different lichen species of different growth forms collected in different locations (Table 3). The sequences showed high similarity with ApMV isolates from apple and pear hosts, and in phylogenetic analysis they were placed in clusters IIa and IIb containing solely isolates from *Maloideae* host (Fig. 3), (Grimová et al. 2013). We have not yet detected ApMV isolates in lichens similar to that from cluster I encompassing isolates from hop, *Prunus*, and other woody tree hosts. Nucleotide and amino acid sequence identity in the capsid protein (CP) gene of the lichen isolates was 94.5–99.6 % and 93.8–100 %, respectively. Sequences of isolates LL4 from *Usnea hirta* and LL5 from *Pseudevernia furfuracea* were identical, although they did not

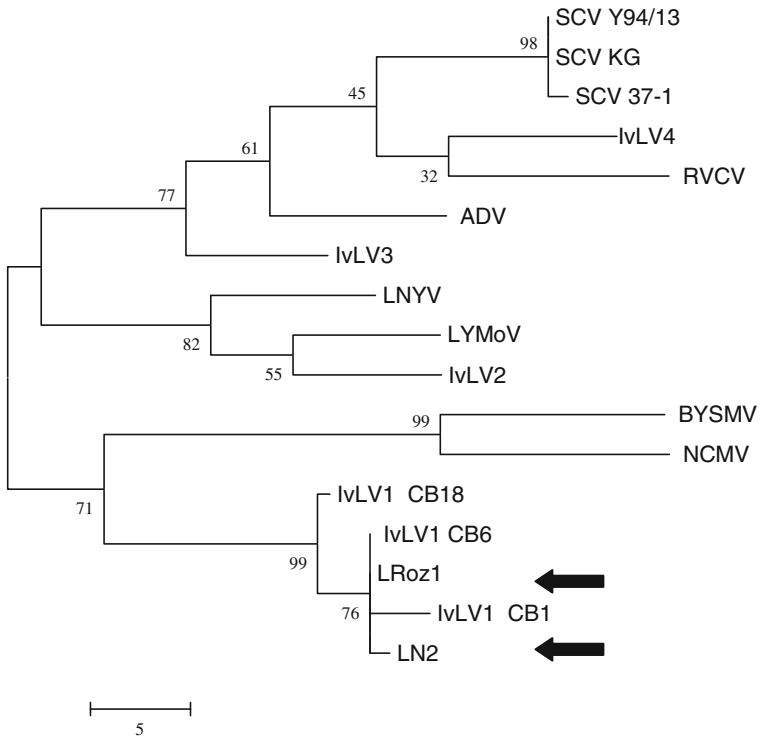

Fig. 1 Phylogenetic reconstruction of partial amino acid sequences of cytorhabdoviruses was done by the maximum parsimony method. The bootstrap values inferred from 1000 replicates are shown next to the branches. Strawberry crinkle virus, isolate KG (SCV-KG, AC number: AY331386); SCV isolate 37–1 (SCV 37–1, AY331387); SCV isolate Y94/13 (SCV Y94/13, AY005146); Raspberry vein chlorosis virus (RVCV, CBL76312); Alfalfa dwarf virus (ADV, HQ380230); Ivy latent virus 4 (IvLV4, JQ650254) IvLV3 (IvLV3, JQ650255), IvLV2 (IvLV2, JQ650253); IvLV1, isolate CB18 (IvLV1 CB18, JQ650256); IvLV1, isolate CB6 (IvLV1, CB6, GQ249163); IvLV1, isolate CB1 (IvLV CB1, Q249162); Lettuce necrotic yellows virus(LNYV, AJ746199); Lettuce yellow mottle virus (LYMoV, EF687738); Barley yellow striate mosaic virus (BYSMV, FJ665628); Northern cereal mosaic virus (NCMV, GU985153); Lichen cytorhabdovirus, isolate LRoz1, (LRoz1, KC109144); Lichen cytorhabdovirus, isolate LN2 (LN2, KC109143)

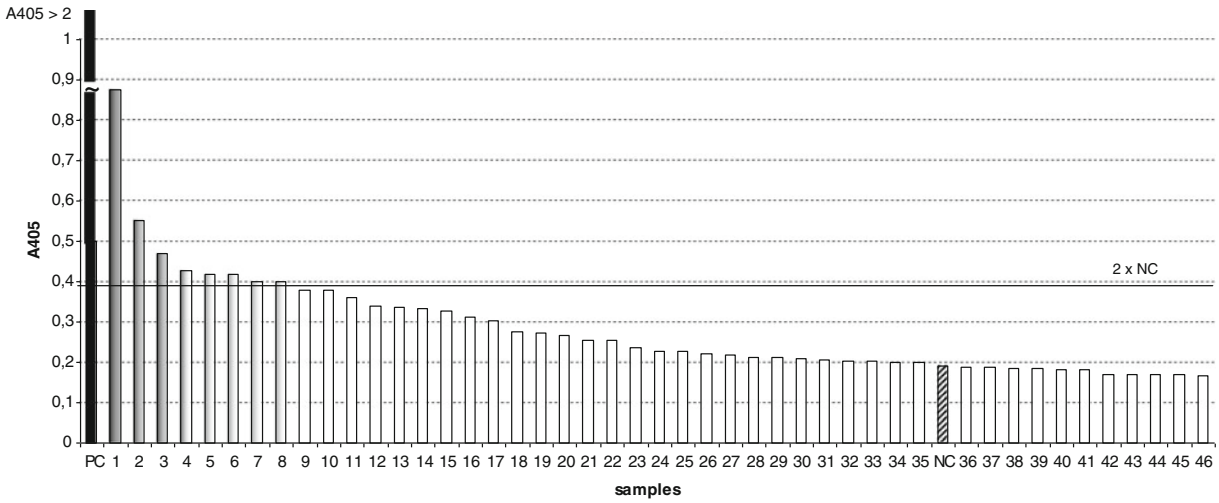

Fig. 2 ApMV DAS-ELISA of 46 lichen samples. Positive (PC) and negative controls (NC) are marked, samples with A_{405} absorbance higher than twice that of negative control are highlighted

contain an identical strain of the *Trebouxia jamesii* photobiont. Both samples were collected from the same birch trunk.

While in the infectivity test no symptoms occurred on inoculated plants, cucumber cotyledons as well as cucumber true leaves were positive in ELISA and in PCR (results not shown).

Localization of ApMV in lichens

The virus could be hosted: (1) in the mycobiont cells (fungal partner), which represent the majority of the lichen thallus; (2) in photobiont cells (algal partner); (3) extracellularly located within lichen thalli; or (4) this can be a surface contamination. We prepared axenic cultures of photobionts identified as *Trebouxia* sp. from the ApMV-positive lichen samples. Cultured photobionts were repeatedly tested by RT-PCR. All cultures were positive for ApMV. The cultivation of axenic mycobionts was unsuccessful, and we therefore conclude that ApMV is present at least in lichen photobionts.

Discussion

The current knowledge of plant virus diversity is far from complete. About 88 % of known plant viruses have been recognized either in cultivated plants or in agricultural weeds, and the majority of the information is derived from symptomatic hosts, but only a small fraction of viruses probably cause diseases (Wren et al. 2006). Wild plants are typically ignored as virus hosts, as

Table 3 Lichen species and their photobionts used in this study

Lichen species	Photobiont	Locality	Acronym	Virus	GenBank accession no.:
Usnea hirta	*Trebouxia jamesii*	birch, Czech Republic	LL4	ApMV	KC469071
Pseudevernia furfuracea	*Trebouxia jamesii*	birch, Czech Republic	LL5	ApMV	KC469072
Xanthoria parietina	*Trebouxia decolorans*	walnut, Czech Republic	LLas1	ApMV	KC4690070
Lasallia pustulata	*Trebouxia simplex*	stone, Norway, Svaner Island	L1B	ApMV	KC469067
Usnea antarctica	*Trebouxia* sp.	stone, James Ross Island, Antarctica	L6B	ApMV	KC469068
Usnea chaetophora	*Trebouxia* sp.	spruce, Norway	LN2	ApMV	KC469069
				rhabdo	KC109143
Cladonia arbuscula	*Trebouxia* sp.	ground, Czech Republic	LRoz1	rhabdo	KC109144

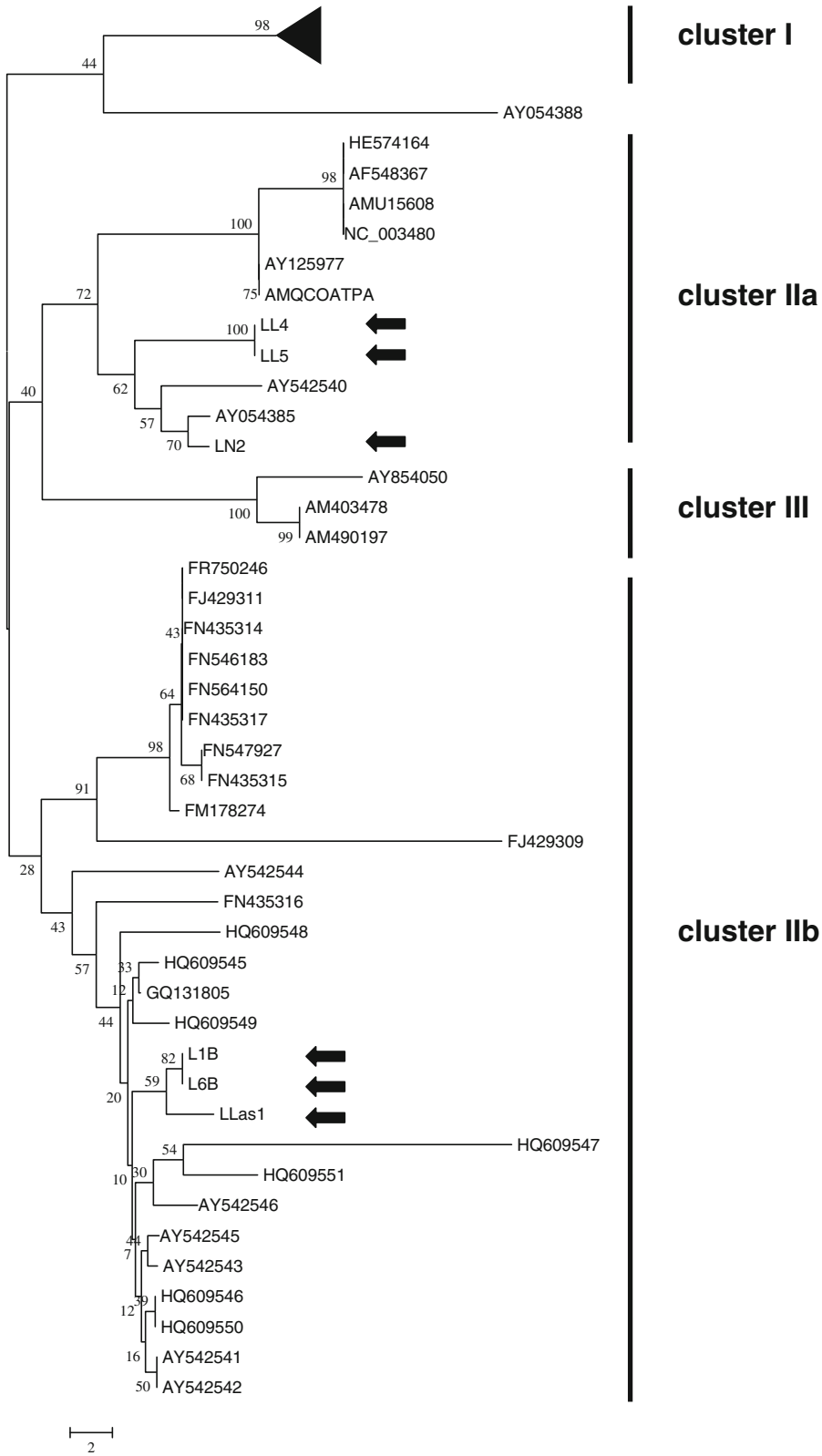

Fig. 3 Phylogenetic reconstruction of partial nucleotide sequences of ApMV was inferred using the maximum parsimony method. The bootstrap values calculated from 1,000 replicates are shown next to the branches. Positions of ApMV lichen isolates are highlighted

are distinct groups of plants, especially some conifers, cycads and ginkgo, ferns and horsetails (Pteridophyta), non-vascular land plants (bryophytes including liverworts, hornworts and mosses), and freshwater algae. In mosses, no special viruses have been found, but antigens of well-known *Tobacco mosaic virus* and *Cucumber green mottle mosaic virus* were detected in two species of Antarctic mosses from the genera *Barbilophozia* and *Polytrichum* (Polischuk et al. 2007). No viruses infecting liverworts are known, but two classes of nucleotide-binding site genes related to plant disease resistance were recently identified in *Marchantia polymorpha* (Xue et al. 2012).

In lichens, as in symbiotic associations, we expected the presence of some mycovirus-like and/or algae-like viruses in at least some of the evaluated samples, but detection of herbaceous plant viruses in lichens was very much unexpected. Plant rhabdoviruses (about 50–100 distinctive species) have been described in a large number of species, but not yet in non-vascular plants. *Apple mosaic virus* is known from apples and pears (*Maloideae* hosts), *Prunus* sp. and hop (Fulton 1972). The only known putative secondary host of ApMV is strawberry (Tzanetakis and Martin 2005). The origin of these two viruses in lichens remains obscure. However, lichens growing on trees hosting ApMV and/or rhabdoviruses could be in proximity with the plant viruses and the way of virus transport/transmission to lichens is not known. We may hypothesize that lichens could be either long-term or only accidental hosts. The second hypothesis, however, could be the more probable, as the sequences of ApMV from the lichen isolates did not differ significantly from the plant isolates. Furthermore, ApMV from lichen thalli retains its ability to infect the known herbaceous host.

It is now merely speculative to consider the significance of potential viruses in lichens, but if they are analogous to viruses of higher plants and fungi, we could expect an influence on lichen viability, stress tolerance, morphology and physiology, as well as influence on gene expressions and other effects. We cannot exclude a control role for viruses in lichens, similar to what has been shown for viruses in aquatic ecosystems, where they control host abundance and host community diversity as well as enable horizontal gene transfer (Wommack and Colwell 2000). A mechanism analogous to mycovirus-mediated hypovirulence (reduction or complete loss of virulence of fungal pathogens as a consequence of virus infection), which play a role in counterbalancing fungal diseases in nature (Nuss 2005), could also be expected in lichens.

We finally suggest that lichens as a whole (or their photobionts, more specifically) could serve as reservoirs for viruses, despite that the way of transmission between different organisms is not clear. Lichens could harbour several viruses simultaneously, as, for example, plant cytorhabdovirus and ApMV were detected in the same host. Last but not least, the presence of viruses could provide impulses to the evolution of the lichen symbionts.

Acknowledgments This work has been conducted with support of Czech Science Foundation project No. P501/12/1747 as well as with institutional support RVO 60077344 from the Biology Centre and RVO 67985939 from the Institute of Botany. The authors are grateful to the CzechPolar (LM2010009) project infrastructure for the possibility to collect and store polar lichen species.

References

Al Rwahnih, M., Daubert, S., Úrbez-Torres, J. R., Cordero, F., & Rowhani, A. (2011). Deep sequencing evidence from single grapevine plants reveals a virome dominated by mycoviruses. *Archives of Virology, 156*, 397–403.

Bates, S. T., Cropsey, G. W. G., Caporaso, G., Knight, R., & Fierer, N. (2011). Bacterial communities associated with the lichen symbiosis. *Applied and Environmental Microbiology, 77*, 1309–1314.

Beckett, R. P., Kranner, I., & Minibayeva, F. V. (2008). In T. H. Nash III (Ed.), *Lichen biology* (2nd ed., pp. 134–151). Cambridge: Cambridge University Press. viii+486 pages.

Cardinale, M., de Castro, J. V., Müller, H., Berg, G., & Grube, M. (2008). In situ analysis of the bacterial community associated with the reindeer lichen Cladonia arbuscula reveals predominance of Alphaproteobacteria. *FEMS Microbiological Letters, 66*, 63–71.

Clauzade, G., Diederich, P., & Roux, C. (1989). Nelikenigintaj fungoj likenlogaj. Ilustrita determinlibro. *Bulletin de la Societe Linneenne de Provence, 1*, 1–142.

Feldman, T. S., Morsy, M. R., & Roossinck, M. J. (2012). Are communities of microbial symbionts more diverse than communities of macrobial hosts? *Fungal Biology, 116*, 465–477.

Friedl, T., & Büdel, B. (2008). In T. H. Nash III (Ed.), *Lichen biology* (2nd ed., pp. 9–26). Cambridge: Cambridge University Press. viii+486 pages.

Fulton, R. W. (1972). *Apple mosaic virus, CMI/AAB descriptions of plant viruses No. 83*. Wellesbourne: Association of Applied Biologists.

Gasulla, F., Guéra, A., & Barreno, E. (2010). A simple and rapid method for isolating lichen photobionts. *Symbiosis, 51*, 175–179.

Ghabrial, S. A., & Suzuki, N. (2010). Fungal viruses. In B. W. J. Mahy (Ed.), *Desk encyclopedia of Plant and fungal virology* (pp. 517–524). Oxford: Academic Press.

Grimová, L., Winkowska, l., Ryšánek, P., Svoboda, P. & Petrzik, K. (2013). Coat protein variability of Apple mosaic virus reflects host preference. *Virus Genes*. doi:10.1007/s11262-013-0925-z.

Guiry, M. D., & Rindi, F. (2005). *AlgaeBase Version 3.0*. Galway: National University of Ireland.

Hibbett, D. S., Binder, M., Bischoff, J. F., Blackwell, M., Cannon, P. F., Eriksson, O. E., et al. (2007). A higher-level phylogenetic classification of the fungi. *Mycological Research, 111*, 509–547.

Hollings, M. (1962). Viruses associated with die-back disease of cultivated mushrooms. *Nature, 196*, 962–965.

King, A.M.Q., Adams, M.J., Carstens, E.B., & Lefkowitz, E.J. (2012). Virus taxonomy, Ninth report of the International Committee on Taxonomy of Viruses, Elsevier Press, San Diego, pp. 1–1327.

Li, W. C., Zhou, J., Guo, S.-Y., & Guo, L.-D. (2007). Endophytic fungi associated with lichens in Baihua mountain of Beijing, China. *Fungal Diversity, 25*, 69–80.

Lumbsch, T., & Huhndorf, D. F. (2009). Outline of Ascomycota-2009. *Fieldiana, 1*, 1–41.

Lutzoni, F., Pagel, M., & Reeb, V. (2001). Major fungal lineages are derived from lichen symbiotic ancestors. *Nature, 411*, 937–940.

Mora, C., Tittensor, D. P., Adl, S., Simpson, A. G. B., & Worm, B. (2011). How many species are there on Earth and in the ocean? *PLoS Biology, 9*(8), e1001127.

Nash, T. H., III. (2008). Lichen sensitivity to air pollution. In T. H. Nash III (Ed.), *Lichen biology* (2nd ed., pp. 299–314). Cambridge: Cambridge University Press. viii+486 pages.

Ng, T. F. F., Duffy, S., Polston, J. E., Bixby, E., Vallad, G. E., & Breitbart, M. (2011). Exploring the diversity of plant DNA viruses and their satellites using vector-enabled metagenomics on whiteflies. *PLoS One, 6*(4), e19050.

Nuss, D. L. (2005). Hypovirulence: mycoviruses at the fungal-plant interface. *Nature Reviews Microbiology, 3*, 632–642.

Petrzik, K. (2012). Bioinformatic analysis of the L polymerase gene leads to discrimination of new rhabdoviruses. *Journal of Phytopathology, 160*, 377–381.

Petrzik, K., & Lenz, O. (2002). Remarkable variability of Apple mosaic virus capsid protein gene after nucleotide position 141. *Archives of Virology, 147*, 1275–1285.

Piercey-Normore, M. D., & DePriest, P. T. (2001). Algal-switching among lichen symbioses. *American Journal of Botany, 88*, 1490–1498.

Polischuk, V., Budzanivska, I., Shevchenko, T., & Oliynik, S. (2007). Evidence for plant viruses in the region of Argentina Islands, Antarctica. *FEMS Microbiology Ecology, 59*, 409–417.

Roossinck, M. J., Saha, P., Wiley, G. B., Quan, J., White, J. D., Lai, H., et al. (2010). Ecogenomics: using massively parallel pyrosequencing to understand virus ecology. *Molecular Ecology, 19*, 81–88.

Rosario, K., Nilsson, C., Lim, Y. W., Ruan, Y., & Breitbart, M. (2009). Metagenomic analysis of viruses in reclaimed water. *Environmental Microbiology, 11*, 2806–2820.

Schwendener, S. (1868). Über die Beziehungen zwischen Algen und Flechtengonidien. *Botanische Zeitung (Berlin)\Bot. Zeitung, 1868*, 289–292.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., & Kumar, S. (2011). MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution, 28*, 2731–2739.

Tao, J. J., Zhou, G. Z., Gui, J. F., & Zhang, Q. Y. (2008). Genomic sequence of mandarin fish rhabdovirus with an unusual small non-transcriptional ORF. *Virus Research, 132*, 86–96.

Tzanetakis, I. E., & Martin, R. (2005). First report of strawberry as a natural host of Apple mosaic virus. *Disease notes, 89*, 431.

White, T. J., Bruns, T., Lee, S., & Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In M. A. Innis, D. H. Gelfand, J. J. Sninsky, & T. J. White (Eds.), *PCR protocols: A guide to methods and applications* (pp. 315–322). New York: Academic Press.

Wilson, W.H., Van Etten, J.L., Schroeder, D.S., Nagasaki, K., Brussaard, C., Delaroque, N., et al. (2012). Phycodnaviridae. In: A.M.Q. King, M.J. Adams, E.B. Carstens, E.J. Lefkowitz (Eds.), Virus Taxonomy: Classification and nomenclature of viruses, Ninth Report of the International Committee of the Taxonomy of Viruses. Elsevier Academic Press, San Diego, pp. 249–262.

Wommack, K. E., & Colwell, R. R. (2000). Virioplankton: viruses in aquatic ecosystems. *Microbiology and Molecular Biology Reviews, 64*, 69–114.

Wren, J. D., Roossinck, M. J., Nelson, R. S., Scheets, K., Palmer, M. W., & Melcher, U. (2006). Plant virus biodiversity and ecology. *PLoS Biology, 4*(3), e80.

Xue, J.-Y., Wang, Y., Wu, P., Wang, Q., Yang, L.-T., Pan, X.-H., et al. (2012). A primary survey on bryophyte species reveals two novel classes of nucleotide-binding site (NBS) genes. *PLoS One, 7*(5), e36700.