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Genetic Diversity of Potassium Ion Channel Proteins Encoded by Chloroviruses That Infect *Chlorella heliozoae*

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Abstract: Chloroviruses are large, plaque-forming, dsDNA viruses that infect chlorella-like green algae that live in a symbiotic relationship with protists. Chloroviruses have genomes from 290 to 370 kb, and they encode as many as 400 proteins. One interesting feature of chloroviruses is that they encode a potassium ion (K^+) channel protein named Kcv. The Kcv protein encoded by SAG chlorovirus ATCV-1 is one of the smallest known functional K^+ channel proteins consisting of 82 amino acids. The Kcv_{ATCV-1} protein has similarities to the family of two transmembrane domain K^+ channel proteins; it consists of two transmembrane α-helixes with a pore region in the middle, making it an ideal model for studying K^+ channels. To assess their genetic diversity, *kcv* genes were sequenced from 103 geographically distinct SAG chlorovirus isolates. Of the 103 *kcv* genes, there were 42 unique DNA sequences that translated into 26 new Kcv channels. The new predicted Kcv proteins differed from Kcv_{ATCV-1} by 1 to 55 amino acids. The most conserved region of the Kcv protein was the filter, the turret and the pore helix were fairly well conserved, and the outer and the inner transmembrane domains of the protein were the most variable. Two of the new predicted channels were shown to be functional K^+ channels.

Keywords: Chloroviruses; potassium ion channels; Kcv channels; algal viruses

1. Introduction

Chloroviruses (family *Phycodnaviridae*) are large, plaque-forming, dsDNA viruses that infect certain chlorella-like green algae that live in a symbiotic relationship with protists [1]. Chloroviruses have an internal membrane and they are icosahedral in shape with a spike structure at one of their vertices [2]. They have genomes that are 290 to 370 kb in size and are predicted to encode up to 400 proteins (CDSs) and 16 tRNAs. These viruses are ubiquitous in nature and have been isolated from freshwater ponds, lakes, and rivers across the globe. There are four groups of chloroviruses based on the host they infect: viruses that infect *Chlorella variabilis* NC64A (referred to as NC64A viruses),

viruses that infect *Chlorella variabilis* Syngen 2-3 (referred to as Osy viruses), viruses that infect *Chlorella heliozoae* SAG 3.83 (referred to as SAG viruses), and viruses that infect *Micratinium conductrix* Pbi, (referred to as Pbi viruses). The most studied chlorovirus is the NC64A virus Paramecium bursaria chlorella virus 1 (PBCV-1); its host, *C. variabilis* NC64A, lives in symbiosis with *Paramecium bursaria*.

The PBCV-1 genome is ~331 kb and encodes 416 predicted CDSs and 11 tRNA genes. About half of the identified CDSs resemble proteins of known function, including some that are novel for a virus. One protein that PBCV-1, as well as most of the chloroviruses, encodes is a potassium ion (K^+) channel protein (named Kcv) [3]. When the 94 amino acid Kcv_{PBCV-1} was discovered, it was the smallest protein known to form a functional K^+ channel. The Kcv_{PBCV-1} protein consists of only the basic functional units that are present in all K^+ channels in that it has a short slide helix, an outer transmembrane helix, a turret, a pore helix, a filter, and an inner transmembrane helix (Figure 1); four of these proteins form a functional K^+ channel.

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                    Slide Helix        TMD1              Turret          Pore Helix
PBCV-1 Kcv      MLVFSKFLTRTEPFMIHLFILAMFVMIYKFFPGGFENNFSVANPDKKASWIDCIYFGVT   59
ATCV-1 Kcv      ----------MLLLIIHIIILIVFTAIYKMLPGGMFSN-------TDPTWVDCLYFSAS   42
                          ::**::** :*. ***::***: .*       .. :*:**:**..:

                    Filter          TMD2
PBCV-1 Kcv      THSTVGFGDILPKTTGAKLCTIAHIVTVFFIVLTL-----  94
ATCV-1 Kcv      THTTVGYGDLTPKSPVAKLTATAHMLIVFAIVISGFTFPW  82
                **:***:**: **:  *** : **:: ** **::
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Figure 1. Kcv_{ATCV-1} sequence alignment with the sequence of the prototype chlorovirus K^+ channel Kcv_{PBCV-1} by ClustalW. The slide helix, outer transmembrane (TMD1) turret, pore helix, selectivity filter, and inner transmembrane (TMD2) of the Kcv_{PBCV-1} are highlighted by the horizontal lines. Asterisks indicate positions which have a fully conserved residue. A colon indicates conservation between amino acids with strongly similar properties. A period indicates conservation between amino acids with less similar properties.

Kcv_{PBCV-1} is hypothesized to play an important role during infection of its host. After the virus attaches to the host cell wall and degrades the wall at the point of attachment, the PBCV-1 internal membrane fuses with the host's plasma membrane [4]. The Kcv channel is located in the virus's internal membrane [5], and once the two membranes are fused, the Kcv channel becomes part of the host membrane. This allows Kcv to participate in the rapid depolarization of the host cell membrane [6,7] and the release of K^+ from the cell [8].

The rapid loss of K^+ from the host and associated water fluxes significantly reduce the host turgor pressure, which aids ejection of viral DNA and virion-associated proteins into the host [9]. Host membrane depolarization also inhibits many host secondary transporters [10] and prevents infection by a second virus [11]. Because of the small size of Kcv, it has served as an excellent model for studying K^+ channels and there are over 60 research publications on Kcv channels.

Since the discovery of Kcv_{PBCV-1}, even smaller chlorovirus-encoded K^+ channel proteins have been described including an 82 amino acid protein from SAG chlorovirus, ATCV-1, referred to as Kcv_{ATCV-1} (Figure 1). Expression studies established that Kcv_{ATCV-1} makes a functional, K^+ selective channel in *Xenopus laevis* oocytes and in yeast [12]. The objective of this study was to isolate and analyze the sequence diversity of the *kcv* gene from 103 SAG chloroviruses that come from freshwater collected throughout the world. Ultimately, one can anticipate some physiological differences among the Kcv channels from the SAG viruses.

2. Materials and Methods

2.1. Cultures

Water samples were collected from lakes, ponds, and rivers from around the United States, Canada, Guatemala, Brazil, Chile, Germany, and Greenland (Table A1 (Appendix A)). The samples were passed

through a 0.45 µm filter (PES filters, Sartorius, Gottingen, Germany). Chloroviruses were isolated from the filtered water samples using a plaque assay on a *C. heliozoae* SAG 3.83 lawn. For the plaque assay, plates were made using Modified Bold's Basal Medium (MBBM) 1.5% agar with tetracycline added at 10 µg/mL [13]. Each plate was filled with about 20 mL of MBBM agar and allowed to solidify. In a tube, 2.5 mL of 0.75% MBBM agar was combined with 1 mL of the filtered water sample and 300 µL of *C. heliozoae* cells (~1.5×10^8 cells/mL). The tube was mixed and poured over the solidified 1.5% MBBM agar plate. Once the top agar layer solidified, the plates were inverted and kept under constant light at 25 °C for a few days. If SAG chloroviruses were present in the water sample, plaques formed on the plates. Two to three unique plaques (e.g., different size and sometimes different shape plaques) were picked from each plate with a sterile toothpick and placed in a 1.5 mL tube filled with *C. heliozoae* cells. These samples were then placed on a spinning wheel for 1 day to propagate the virus. The resulting lysates were serially diluted to 10^{-6} in virus suspension buffer (VSB, 50 mM Tris HCl, 10 mM $MgCl_2$, pH 7.8) and 100 µL of the resultant dilution was plaqued. Each virus sample was plaque-purified two or three times to ensure that one had a single virus. For the PCR DNA template preparation, 100 µL of viral lysate was boiled in deionized sterile water for 5–10 min.

2.2. Primer Selection

DNA sequences 450 to 500 bp upstream and 230 to 350 bp downstream from the *kcv* gene from 13 previously sequenced SAG chloroviruses [14] were used to identify conserved regions for designing degenerate primers. Conserved regions were identified inside the aligned sequences, and four forward primers and five reverse primers (Table 1) were made and tested using known SAG virus DNAs (ATCV-1, BRO604, Can0610, Canal1, GM0701, MN0810, MO0605, NEJV2, NTS1, OR0704, TN603, WI0606). The primers that identified all of the *kcv* genes were forward primer Kcv8 Frw and reverse primer Kcv6 Rvs as well as forward primer Kcv9 Frw and reverse primer Kcv6 Rvs. As a result, the primer set Kcv9/Kcv6 was selected to amplify the *kcv* genes.

Table 1. Primers tested to isolate the *kcv* genes.

	Name	Sequence	Position	GC Content (%)	T_m (°C)
Forward Primers	Kcv6 Frw	CTT TAG YYT TYY TCK GVC	−366	34	49
	Kcv7 Frw	CTT TAG YYT TYY TCK GVC G	−366	38	54
	Kcv8 Frw	GAA GCA GGY ACC ACT TTA G	−379	47	53
	Kcv9 Frw	GCA GGY ACC ACT TTA G	−376	50	47
Reverse Primers	Kcv6 Rvs	CRC RGM ATR TRT CAT TTG WCC C	+256	48	64
	Kcv7 Rvs	CTT ACR CRG MAT RTR TCA TTT G	+259	39	56
	Kcv8 Rvs	CTT ACR CRG MAT RTR TC	+264	44	44
	Kcv9 Rvs	HKB YMC GAT CTT ATA CAC	+292	39	45
	Kcv10 Rvs	CAT TTC TTA CRC RGM ATR TRT C	+264	39	55

2.3. Polymerase Chain Reactions (PCR)

The conditions for the PCR reactions followed the recommendations of New England Biolab's (Beverly, MA, USA) Phusion High Fidelity DNA Polymerase kit. The 50 µL PCR reactions contained 10 µL of 5x Phusion High Fidelity buffer, 1 U Phusion DNA polymerase, and 1 µL of DNA template. The final concentration of dNTPs in the PCR reaction was 0.2 mM and the primer final concentration was 0.5 µM forward primer and 0.5 µM reverse primer. The reactions were run for 5 min at 94 °C, followed by 35 cycles of 1 min at 94 °C, 30 s at 56 °C, 1 min at 72 °C, and at the end the final extension 15 min at 72 °C. Deionized water was used as a negative control.

The PCR products were gel-purified by mixing 20 µL of an amplified sample with a small amount of Ficoll gel loading buffer and loaded on a 1% agarose gel. The New England Biolabs log-2 ladder was used as a molecular weight marker. The gel was run at 5 V/cm for 1 h, then imaged by exposing the gel to ultraviolet light. Amplified *kcv* genes were excised from the gel and the DNA extracted using the QIAquick Gel Extraction Kit following the manufacturer's instruction (QIAGEN Hilden, Germany).

The purified DNAs were sequenced using Sanger sequencing by a commercial provider. The accession numbers for the *kcv* sequences in GenBank are MT560092-MT560194.

2.4. Phylogenetic Analysis

The *kcv* gene DNA sequences were translated into amino acid sequences, and all of the unique Kcv proteins were aligned. Geneious 11.0.5 software (Biomatters Ltd., Auckland, New Zealand, https://www.geneious.com) was used for the DNA and protein sequence alignment (Geneious Alignment with the default settings) and the phylogenetic tree was constructed with PhyML (version 3.3.20180621), which is Maximum likelihood, using the default settings.

2.5. Functional Reconstitution of kcv Genes in Planar Lipid Bilayers

K^+ channel proteins were translated in vitro into nanodiscs (NDs) with the MembraneMax HN Protein Expression Kit (Invitrogen, Carlsbad, CA, USA) as described previously [15]. A His-tag attached to the scaffold protein of the NDs allowed purification of channel/ND-complexes via metal chelate affinity chromatography. To eliminate unspecific binders, the column was washed three times with 400 µL of a 20 mM imidazole solution. Finally, the His-tagged NDs were eluted in three fractions with 200 µL of a 250 mM imidazole solution. All centrifugation steps were performed at 700 g for 2 min.

Single-channel recordings were done with a vertical bilayer set up (IonoVation, Osnabrück, Germany) as described previously [16]. The experimental solution contained 100 mM KCl and was buffered to pH 7.0 with 10 mM HEPES/KOH. As a lipid, we used 1,2-diphythanoyl-*sn*-glycero-3-phosphocholine (DPhPC) (Avanti Polar Lipids, Alabaster, AL, USA) at a concentration of 15 mg/mL in n-pentane (MERCK KGaA, Darmstadt, Germany).

3. Results

3.1. kcv Genes Selected for Analysis

In total, *kcv* genes from 83 SAG chloroviruses were obtained from the PCR experiments. In addition, the *kcv* genes from the 13 SAG chloroviruses that had been sequenced previously [14] and *kcv* genes from 7 recently sequenced SAG chloroviruses (not yet in the database) were included in the analysis. Thus, in total we compared *kcv* genes from 103 SAG chloroviruses (Table A1). Overall, these viruses were from three continents and seven countries.

3.2. Diversity of kcv Genes

Alignment of the nucleotide sequences indicated that the 103 *kcv* genes had substitutions in 125 of the 249 nucleotides (~50%) (assuming that all the proteins were 82 amino acids long (but see below)) producing 42 unique DNA sequences (Figure A1 (Appendix B)). Nine of the viruses with identical *kcv* DNA sequences included a virus isolated in Germany in 2002 and viruses isolated in various parts of the United States of America 4 to 16 years later. Twenty of the virus isolates with identical *kcv* DNA sequences were from ponds near Hohenheim, Germany that were collected on the same day in 2017. It is important to note that the type SAG virus, ATCV-1, was isolated from one of these ponds in 2002. The sequences of the newly isolated viruses differed from ATCV-1 virus by 18–21 amino acids. In these same recent German collections, there were viruses with three additional *kcv* DNA sequences. The *kcv* DNA sequence from 18 of the 103 viruses was only found one time.

3.3. Diversity of the Kcv Proteins

The 42 unique *kcv* DNA sequences produced 26 unique proteins (Figure 2). Of the 26 unique protein sequences, 18 of them were 82 amino acids long, 4 were 84 amino acids, 2 were 85 amino acids, 1 was 87 amino acids, and 1 was 89 amino acids. The proteins with 84 and 85 amino acids had either 2 or 3 extra amino acids at their C-terminal ends. The 87 amino acid Kcv protein from a virus isolate

from New York state had 5 amino acids added to the N-terminus of the protein. However, this protein has an internal Met that would create an 82 amino acid protein and so we suspect that this internal AUG is the actual translation start site. The 89 amino acid protein from a virus, GLND22, isolated in Greenland has 8 extra amino acids in the turret region between the outer transmembrane domain and the pore region. This turret region also has extra amino acids in the slightly larger Kcv proteins from the NC64A viruses (Figure 1) and the Pbi viruses [17].

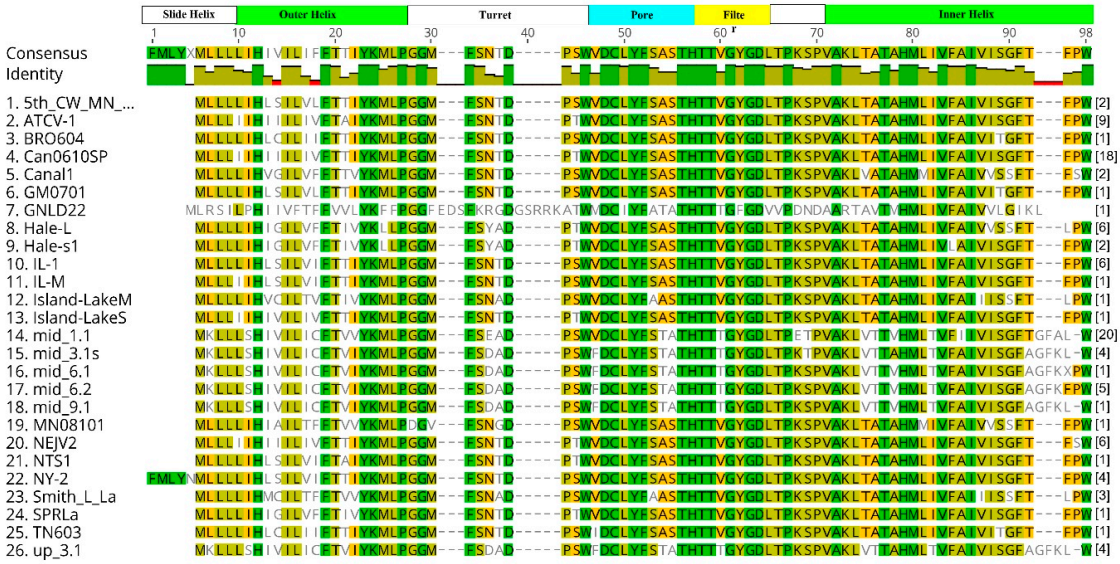

Figure 2. Amino acid alignment of SAG chlorovirus unique Kcv proteins using Geneious 11.0.5 software. The number in brackets at the end of each protein is the number of times that the protein had an identical sequence of the 103 viruses examined. Score matrix is Identity. Green color denotes identical amino acids. Other shades of amino acids indicate a level of conservation from olive color as being the most conserved to white color not conserved.

The $Kcv_{Can0610SP}$ only differed from Kcv_{ATCV-1} by one amino acid. The Kcvs from viruses collected from Germany in 2017 differed from $Kcv_{ATCV-1,}$ which originally came from Germany, by 18 to 21 amino acids. The Kcv_{GNLD22} differed the most from Kcv_{ATCV-1} with 55 amino acid differences or 56% of the amino acids. The remaining virus Kcvs differed from Kcv_{ATCV-1} by 2 to 13 amino acids.

Alignment of the 26 unique proteins revealed that some areas of the Kcv protein were more conserved than others. The filter domain is typically the most highly conserved domain in K^+ channel proteins and 20 of the 26 proteins had a TTVGYGDL sequence. Five of the remaining six Kcvs had a TTTGYGDL sequence and the remaining Kcv, Kcv_{GLND22} from Greenland, had a TTTGFGDV sequence (Figure 2). All the SAG chlorovirus Kcvs essentially lack an N-terminal slide helix domain (Figure 1).

A phylogenetic tree of the 26 Kcv proteins resulted in 3 major clades (Figure 3). The largest clade had 19 Kcv proteins from viruses primarily collected across the United States but also included viruses isolated in Canada, Guatemala, and Brazil, and one from Germany. Kcv proteins isolated from the recent German water samples formed a separate cluster with a distance of 0.4925 substitutions from the rest of the SAG Kcvs (Figure 3). The Kcv_{GNDL22} from Greenland also formed a distinct clade with a distance of 1.518 substitutions from the German samples and a distance of 1.6675 substitutions from all the rest. Interestingly, Kcv_{GNDL22} had more similarity to a Kcv from a Pbi virus MT325 than it did to the other SAG viruses (Figure 3). Virus GNDL22 is also interesting because about 15% of its CDSs are more similar to the MT325 Pbi virus and the other 85% are most similar to SAG viruses. Thus, GNDL22 appears to be some type of hybrid virus.

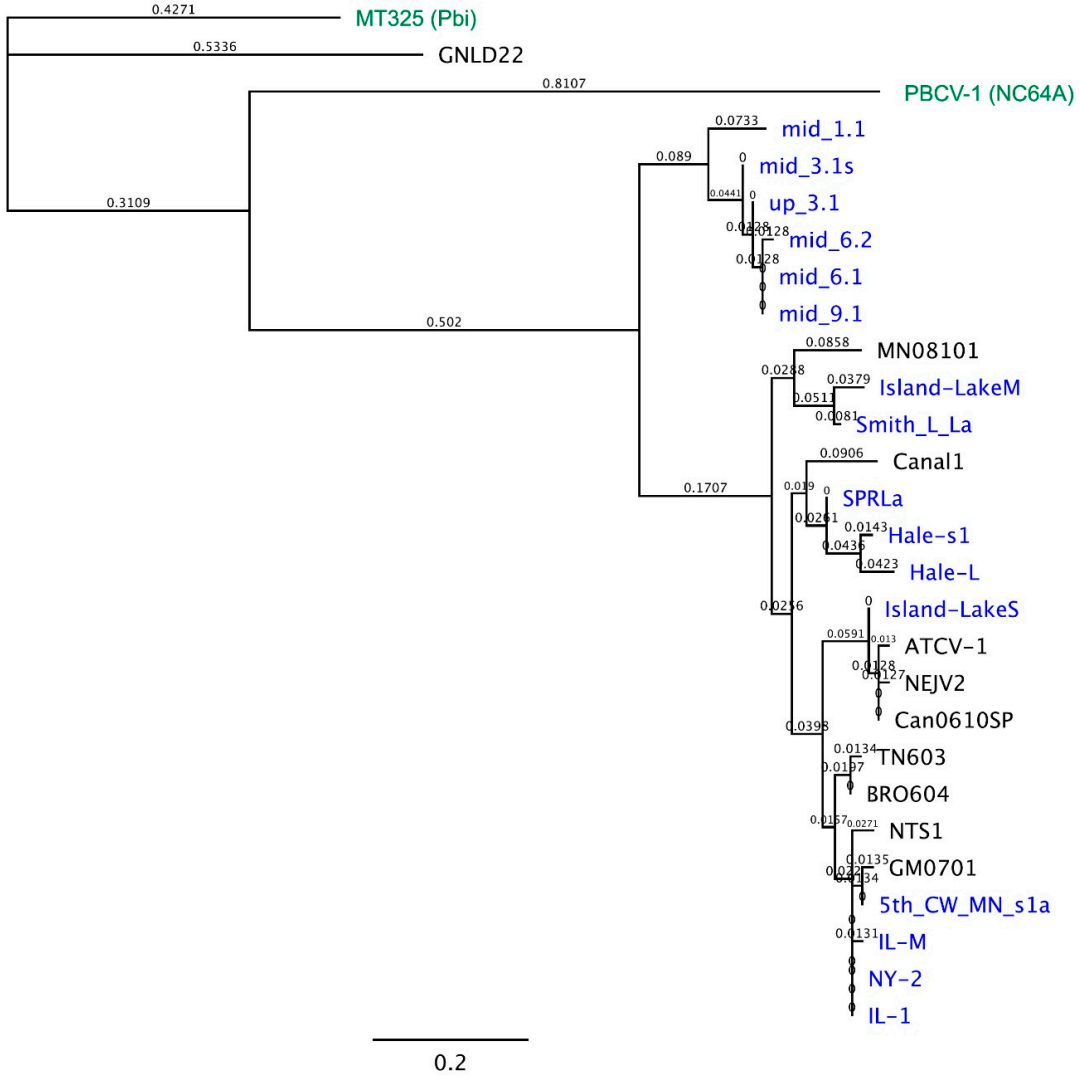

Figure 3. Phylogenetic tree of Kcv proteins from 26 SAG chloroviruses. PhyML, which is Maximum likelihood, with the default settings was used to construct the tree. The branch length shows dissimilarity between strains and the values on the branches are the number of changes. The viruses in blue represent the new Kcv proteins reported in this manuscript. Viruses MT325 representing a Pbi virus and PBCV-1 representing an NC64A virus are in green. Additional information on each of the Kcv proteins from the SAG viruses, including where they were isolated and the number of times that protein sequence appeared, is included in Table A1.

3.4. Functional Reconstitution of Two New Kcv Channels in Planar Lipid Bilayers

To test whether the newly discovered genes were coding for functional K^+ channels, we selected two representatives for functional testing. The putative channel proteins Kcv_{GNLD22} and $Kcv_{Can0610SP}$ were translated in vitro into nanodiscs and after purification reconstituted in planar lipid bilayers. The electrical recordings in Figure 4 show that the two proteins generated typical single-channel fluctuations at positive and negative voltages in the presence of 100 mM KCl on both sides of the membrane. These experiments established that the two genes code for functional ion channels. The overall properties of the two new channels were similar to those of Kcv_{NTS} (Figure 4), a well-studied representative of the K^+ channels from SAG viruses [18]; Kcv_{NTS} differed from the reference channel Kcv_{ATCV-1} by four amino acids. Kcv_{GNLD22} and $Kcv_{Can0610SP}$ were selected because of their large (55 amino acids) and small (1 amino acid) deviation from the reference channel Kcv_{ATCV-1}. All three channels exhibited a hallmark of the chlorovirus K^+ channels with well-resolved channel openings at

positive voltages and flicker type gating at negative voltages. This flicker type gating resulted from very fast open/close transitions at negative voltages, which cannot be fully resolved by the recording equipment. As a consequence, the unitary channel conductance exhibited an apparent decrease at negative voltages [19,20]. However, it is interesting to note that this fast gating was already apparent in Kcv_{GNLD22} at voltages negative of 0 mV while the negative slope in the two other channels only occurred at voltages more negative than about -100 mV. A closer scrutiny of the single-channel data also showed additional differences between the three channels. Comparison of the unitary channel conductance showed that this value in Kcv_{GNLD22} (45 ± 3 pS, n = 7) was only half as big as in Kcv_{NTS} (87 ± 1.4 pS, n = 9) and $Kcv_{Can0610SP}$ (110 ± 3, n = 9). Another striking feature of Kcv_{GNLD22} was a strong voltage-dependent decrease in open probability at positive voltages, which was not seen in the other two. A peculiar feature of $Kcv_{Can0610SP}$ was long-lived closed states at negative voltages, which explains a voltage-dependent decrease in open probability at negative voltages. These long closures were absent in the two other channels; in Kcv_{GNLD22} it was even difficult to observe any distinct closure at negative voltages.

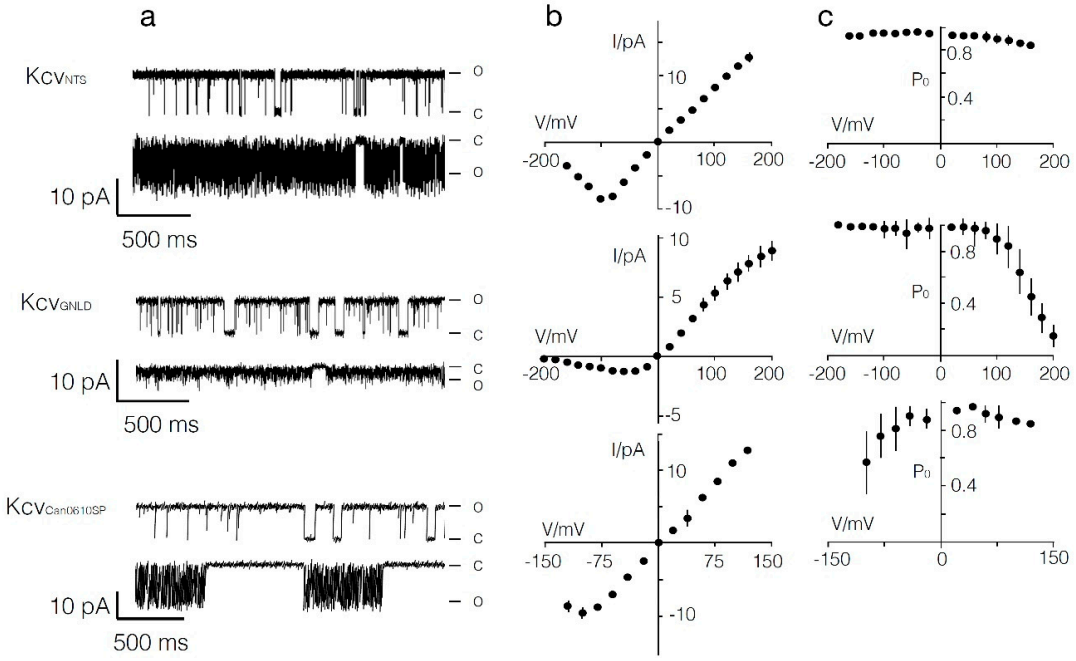

Figure 4. Two of the newly discovered K^+ channels are active. Characteristic single-channel fluctuations (**a**), mean single-channel I/V relations (**b**), and mean open probabilities (**c**) of Kcv_{NTS} (top row), Kcv_{GNLD22} (middle row), and $Kcv_{Can0610SP}$ (bottom row) at ±120 mV. The closed (c) and open (o) levels are indicated along the current traces. Data are means ±s.d. from ≥ 3 independent recordings of channels in the same row. Data were recorded in a DPhPC bilayer with symmetrical 100 mM KCl, 10 mM HEPES, pH 7 in cis and trans chamber.

4. Discussion

This manuscript demonstrated that the *kcv* gene is ubiquitous among the 103 chloroviruses that infect *C. heliozoae* SAG 3.83, which resulted in 42 unique *kcv* DNA sequences. The 42 unique *kcv* DNA sequences produced 26 unique proteins or 26 new Kcv channels. Using the Kcv_{ATCV-1} channel as representative of the SAG viruses, the Kcv_{ATCV-1} differed from the Kcv_{GNLD22} channel from Greenland by 55 amino acids or 56% of their amino acids. Other channels differ from the Kcv_{ATCV-1} channel by 1 to 21 amino acids.

Due to the role K^+ channels play in chlorovirus infection and reproduction by depolarizing the host cell membrane, it is not surprising that *kcv* genes were found in all of the samples [19]. Therefore, we predict that all of the amino acid substitutions in the channels from the SAG viruses will produce

functional channels; in fact, the two channels that were tested were functional (Figure 4). Furthermore, we predict that the SAG Kcv channels may have some different biophysical properties, especially the Kcv coded by the GNDL22 virus. This prediction is confirmed by scrutiny of the functional properties of the two new channels and a comparison with the well-studied SAG-type channel Kcv_{NTS} [18]. The mutual comparisons identified differences in the unitary conductance and gating between the different channels. The apparent impact of a few amino acid exchanges between the proteins on functional properties is in good agreement with previous investigations in which we found that mutation of the common Gly at the end of the second transmembrane domain in Kcv_{NTS} to Ser introduces one distinct gate with a long-lived close time [18]. Several of the new channels have the same critical Ser in the same position (90 in Figure 2). Thus, a functional analysis of these channels will be interesting.

The data are also in line with a previous study where the *kcv* gene was sequenced from 41 NC64A viruses, including the prototype chlorovirus PBCV-1. Sixteen of the 94 amino acids in the NC64A Kcv protein differed resulting in six new Kcv channels [20]. The six Kcv-like channels, which differed from Kcv_{PBCV-1} by 4 to 12 amino acids, produced K^+ selective currents in *Xenopus laevis* oocytes with altered biophysical properties, including current kinetics, voltage dependency, and inhibition by Cs^+ [20,21]. The amino acid changes together with the different properties observed in the six Kcv-like channels were used to guide site-directed mutations, either singularly or in combination, to identify key amino acids that confer specific properties to Kcv [20,21].

While we assume that the chloroviruses require Kcv activity to replicate, we do have to mention that out of the more than 150 chloroviruses that have been examined for a *kcv* gene, two NC64A viruses either lack a *kcv* gene or have a truncated form [1]. We would like to disrupt the *kcv* gene in some of the chloroviruses to see what effect this has on virus replication, however, currently the technology to do this experiment is not available.

Compared with the larger Kcv_{PBCV-1}, the 82 amino acid Kcv_{ATCV-1} lacks a cytoplasmic N-terminus, that is, the slide helix region and a number of charged amino acids in its turret domain (Figure 1) [12]. The only known K^+ channel proteins smaller than Kcv_{ATCV-1} are two channels that are encoded by viruses that infect small marine algae in the *Micromonas* genus [22]. The channel $Kmbv_1$ is 79 amino acids long and $Kmpv_{12T}$ is 78 amino acids long. Expression of $Kmbv_1$ in HEK293 cells results in currents. However, expression of $Kmpv_{12T}$ in the same cells does not produce a current, but it does produce a current in a planar lipid bilayer [22]. All of these virus-encoded channels have a long evolutionary history and probably have a common evolutionary ancestor.

In summary, *kcv* genes from 103 geographically distinct SAG viruses were sequenced to assess their genetic diversity. Of the 103 *kcv* genes, there were 42 unique DNA sequences that translated into 26 new Kcv proteins, which we predict will have some different biophysical properties. The amino acid changes together with the expected different properties will be used to guide site-directed mutations to identify key amino acids that confer specific properties to Kcv.

Author Contributions: Conceptualization, J.L.V.E. and G.T.; methodology, C.R.M. and I.V.A.; software, C.R.M. and I.V.A.; validation, C.R.M., J.L.V.E., I.V.A., J.S.G., R.M.C., G.T., and B.H.; formal analysis, C.R.M. and G.T.; investigation, C.R.M., F.C.F., K.K., O.R., and B.H.; resources, C.R.M., I.V.A., F.C.F., J.S.G., R.M.C., and G.T.; data curation, C.R.M. and G.T.; writing—original draft preparation, C.R.M. and J.L.V.E.; writing—review and editing, C.R.M., J.L.V.E., I.V.A., R.C.C., F.C.F., J.S.G., G.T., B.H.; visualization, C.R.M., I.V.A., G.T.; supervision, J.L.V.E. and G.T.; project administration, J.L.V.E.; funding acquisition, J.L.V.E. and G.T. All authors have read and agreed to the published version of the manuscript.

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Appendix A

Table A1. Source and sequence of SAG chlorovirus encoded potassium ion channel (Kcv) genes [1].

Sample ID	DNA Sequence	Location collected	Date collected	DNA Group	Amino Acid Group
5th_CW_MN_s1a (2) [2]	ATGTTGCTGCTTCTCATACATCTCAGCATTTTGGT ACTTTTCACTACCATATACAAGATGCTCCCCGGTGGC ATGTTCTCGAACACGGATCCGTCCTGGGTCGATTGCC TGTACTTTTCGGCATCAACGCACACCACCGTGGGGTA CGGGGACCTCACGCCAAAATCACCCGTGGCAAAACT CACGGCCACGGCACACATGCTGATCGTATTCGCGATC GTCATTTCTGGCTTCACGTTCCCGTGGTAA	5th Crow Wing Lake; Nevis, Minnesota	5/4/2017	5th_CW_MN_s1a	5th_CW_MN_s1a
5th_CW_MN_L4	ATGTTGCTGCTTCTCATACATCTCAGCATTTTGGTA CTTTTCACTACCATATACAAGATGCTCCCCGGTGGCAT GTTCTCGAACACGGATCCGTCCTGGGTCGATTGCCTGT ACTTTTCGGCATCAACGCACACCACCGTGGGGTACGGG GACCTCACGCCAAAATCACCCGTGGCAAAACTCACGGC CACGGCACACATGCTGATCGTATTCGCGATCGTCATTTCT GGCTTCACGTTCCCGTGGTAA	5th Crow Wing Lake; Nevis, Minnesota	5/4/2017	5th_CW_MN_s1a	5th_CW_MN_s1a
ATCV-1 (8) [9]	ATGTTGCTGCTTATCATACATATCATCATTCTGATA GTGTTCACTGCCATCTACAAGATGCTCCCCGGC GGCATGTTCTCGAACACAGACCCTACTTGGGTT GATTGCCTGTACTTTTCGGCATCGACGCACACC ACCGTGGGGTACGGAGATCTCACGCCCAAATC ACCCGTGGCAAAACTCACGGCAACGGCACAC ATGTTGATCGTATTCGCGATCGTCATTTCTGGCT TCACGTTTCCGTGGTAG	Germany	2002	ATCV-1	ATCV-1
MO0605SPH	ATGTTGCTGCTTATCATACATATCATCATTCTGATA GTGTTCACTGCCATCTACAAGATGCTCCCCGGCG GCATGTTCTCGAACACAGACCCTACTTGGGTTGA TTGCCTGTACTTTTCGGCATCGACGCACACCACC GTGGGGTACGGAGATCTCACGCCCAAATCACCCG TGGCAAAACTCACGGCAACGGCACACATGTTGAT CGTATTCGCGATCGTCATTTCTGGCTTCACGTTTCCGTGGTAG	Missouri	2006	ATCV-1	ATCV-1
WI0606	ATGTTGCTGCTTATCATACATATCATCATTCTGATA GTGTTCACTGCCATCTACAAGATGCTCCCCGGCG GCATGTTCTCGAACACAGACCCTACTTGGGTTGA TTGCCTGTACTTTTCGGCATCGACGCACACCACC GTGGGGTACGGAGATCTCACGCCCAAATCACCCG TGGCAAAACTCACGGCAACGGCACACATGTTGAT CGTATTCGCGATCGTCATTTCTGGCTTCACGTTTCCGTGGTAG	Madison, Wisconsin	2006	ATCV-1	ATCV-1

Table A1. *Cont.*

Sample ID	DNA Sequence	Location collected	Date collected	DNA Group	Amino Acid Group
Drexel	ATGTTGCTGCTTATCATACATATCATCATTCTG ATAGTGTTCACTGCCATCTACAAGATGCTCCCCGGC GGCATGTTCTCGAACACAGACCCTACTTGGGTTGAT TGCCTGTACTTTTCGGCATCGACGCACACCACCGTG GGGTACGGAGATCTCACGCCCAAATCACCCGTGGC AAAACTCACGGCAACGGCACACATGTTGATCGTATT CGCGATCGTCATTTCTGGCTTCACGTTTCCGTGGTAG	Drexel, Missouri	Jun-17	ATCV-1	ATCV-1
NPRLb	ATGTTGCTGCTTATCATACATATCATCATTCTGATA GTGTTCACTGCCATCTACAAGATGCTCCCCGGCGGC ATGTTCTCGAACACAGACCCTACTTGGGTTGATTG CCTGTACTTTTCGGCATCGACGCACACCACCGTG GGGTACGGAGATCTCACGCCCAAATCACCCGTGG CAAAACTCACGGCAACGGCACACATGTTGATCGT ATTCGCGATCGTCATTTCTGGCTTCACGTTTCCGTGGTAG	North Platte River; Highway 27, Nebraska	10/23/2017	ATCV-1	ATCV-1
SPRma	ATGTTGCTGCTTATCATACATATCATCATTCTGAT AGTGTTCACTGCCATCTACAAGATGCTCCCCGG CGGCATGTTCTCGAACACAGACCCTACTTGGGT TGATTGCCTGTACTTTTCGGCATCGACGCACAC CACCGTGGGGTACGGAGATCTCACGCCCAAAT CACCCGTGGCAAAACTCACGGCAACGGCACA CATGTTGATCGTATTCGCGATCGTCATTTCTGG CTTCACGTTTCCGTGGTAG	South Platte River; Big Spring, Nebraska	10/23/2017	ATCV-1	ATCV-1
SPRsb	ATGTTGCTGCTTATCATACATATCATCATTCTG ATAGTGTTCACTGCCATCTACAAGATGCTCCCCGG CGGCATGTTCTCGAACACAGACCCTACTTGGGTT GATTGCCTGTACTTTTCGGCATCGACGCACACCA CCGTGGGGTACGGAGATCTCACGCCCAAATCAC CCGTGGCAAAACTCACGGCAACGGCACACATG TTGATCGTATTCGCGATCGTCATTTCTGGCTTCA CGTTTCCGTGGTAG	South Platte River; Big Spring, Nebraska	10/23/2017	ATCV-1	ATCV-1

Table A1. *Cont.*

Sample ID	DNA Sequence	Location collected	Date collected	DNA Group	Amino Acid Group
Smith-Lake_ Large-Plaque	ATGTTGCTGCTTATCATACATATCATCATTCTG ATAGTGTTCACTGCCATCTACAAGATGCTCCCCGG CGGCATGTTCTCGAACACAGACCCTACTTGGGTT GATTGCCTGTACTTTTCGGCATCGACGCACACCA CCGTGGGGTACGGAGATCTCACGCCCAAATCAC CCGTGGCAAAACTCACGGCAACGGCACACATG TTGATCGTATTCGCGATCGTCATTTCTGGCTTCA CGTTTCCGTGGTAG	Smith Lake, Western Nebraska	2018	ATCV-1	ATCV-1
OH-S (1)	ATGTTGCTGCTTATCATACATATCATCATTCTGA TAGTGTTCACTGCCATCTACAAGATGCTCCCCGGCG GCATGTTCTCGAACACAGACCCTACTTGGGTTGATT GCCTGTACTTTTCGGCATCGACGCACACCACCGT GGGGTACGGAGATCTCACGCCCAAATCACCCGT GGCAAAACTCACGGCAACGGCACACATGTTGAT CGTATTCGCGATCGTCATTTCTGGCTTCACATTT CCGTGGTAG	Ohio	Jul-17	OH-S	ATCV-1
BRO604 (1) [1]	ATGTTGCTGCTTCTCATACACCTCTGTATTCTGAT AATTTTTACTACCATATACAAGATGTTGCCCGGAGG CATGTTCTCTAACACGGACCCGTCGTGGGTCGATTG CCTGTACTTCTCGGCATCAACGCACACCACCGTGGG GTACGGGGATCTCACGCCCAAATCACCCGTGGCAA AACTCACAGCAACGGCACACATGCTGATCGTATTCG CGATCGTAATAACTGGCTTCACATTCCCGTGGTAA	Brazil	2006	BRO604	BRO604
Can0610SP (2) [18]	ATGTTGCTGCTTATCATACATATCATCATTCTGATA GTGTTCACTACCATCTACAAGATGCTCCCCGGCGG CATGTTCTCGAACACAGACCCTACTTGGGTTGATT GCCTGTACTTTTCGGCATCGACGCACACCACTGT GGGGTACGGAGATCTCACGCCCAAATCACCCGT GGCAAAACTCACGGCAACGGCACACATGTTGA TCGTATTCGCGATCGTCATTTCCGGCTTCACG TTCCCGTGGTAG	British Columbia, Canada	2006	Can0610SP	Can0610SP

Table A1. *Cont.*

Sample ID	DNA Sequence	Location collected	Date collected	DNA Group	Amino Acid Group
Or0704	ATGTTGCTGCTTATCATACATATCATCATTCT GATAGTGTTCACTACCATCTACAAGATGCTCCCCGGC GGCATGTTCTCGAACACAGACCCTACTTGGGTTGAT TGCCTGTACTTTTCGGCATCGACGCACACCACTGTG GGGTACGGAGATCTCACGCCCAAATCACCCGTGGC AAAACTCACGGCAACGGCACACATGTTGATCGTA TTCGCGATCGTCATTTCCGGCTTCACGTTCCCGTGGTAG	Willamette River; Corvallis, Oregon	Jul-07	Can0610SP	Can0610SP
Chile_7s (1)	ATGTTGCTGCTTATCATACATATCATCATTCTGATA GTGTTCACTACCATATACAAGATGCTCCCCGGCGGCATG TTCTCGAACACAGACCCTACTTGGGTTGATTGCCTGTACT TTTCGGCATCGACGCACACCACTGTGGGGTACGGAGATCT CACGCCCAAATCACCCGTGGCAAAACTCACGGCAACGGCA CACATGTTGATCGTATTCGCGATCGTCATTTCCGGCTT CACGTTCCCGTGGTAG	Chile	Jan-19	Chile_7s	Can0610SP
K2 (5)	ATGTTGCTGCTTATCATACATATCATCATTCTGATAGTGTTC ACTACCATCTACAAGATGCTCCCCGGTGGCATGTTCTCGAACA CGGACCCGACTTGGGTTGATTGCCTGTACTTTTCGGCATCGA CGCACACCACCGTGGGGTACGGAGATCTCACGCCCAAATC ACCCGTGGCAAAACTCACGGCAACGGCACACATGTTGAT CGTATTCGCGATCGTCATTTCCGGCTTCACGTTCCCGTGGTAG	Missouri River; Atchison, Kansas	5/31/2017	K2	Can0610SP
K2s	ATGTTGCTGCTTATCATACATATCATCATTCTGATAGTGT TCACTACCATCTACAAGATGCTCCCCGGTGGCATGTTCTCGA ACACGGACCCGACTTGGGTTGATTGCCTGTACTTTTCGGCAT CGACGCACACCACCGTGGGGTACGGAGATCTCACGCCCAA ATCACCCGTGGCAAAACTCACGGCAACGGCACACATGTTG ATCGTATTCGCGATCGTCATTTCCGGCTTCACGTTCCCGTGGTAG	Missouri River; Atchison, Kansas	5/31/2017	K2	Can0610SP

Table A1. *Cont.*

Sample ID	DNA Sequence	Location collected	Date collected	DNA Group	Amino Acid Group
NPRma	ATGTTGCTGCTTATCATACATATCATCATTCTGATAGTGTTC ACTACCATCTACAAGATGCTCCCCGGTGGCATGTTCTCG AACACGGACCCGACTTGGGTTGATTGCCTGTACTTTTC GGCATCGACGCACACCACCGTGGGGTACGGAGATCTC ACGCCCAAATCACCCGTGGCAAAACTCACGGCAACG GCACACATGTTGATCGTATTCGCGATCGTCATTTCCG GCTTCACGTTCCCGTGGTAG	North Platte River; Highway 27, Nebraska	10/23/2017	K2	Can0610SP
NPRsb	ATGTTGCTGCTTATCATACATATCATCATTCTGATAGTGTTC ACTACCATCTACAAGATGCTCCCCGGTGGCATGTTCTCGA ACACGGACCCGACTTGGGTTGATTGCCTGTACTTTTCGG CATCGACGCACACCACCGTGGGGTACGGAGATCTCACG CCCAAATCACCCGTGGCAAAACTCACGGCAACGGCAC ACATGTTGATCGTATTCGCGATCGTCATTTCCGGCTTC ACGTTCCCGTGGTAG	North Platte River; Highway 27, Nebraska	10/23/2017	K2	Can0610SP
Pl_R_Lma	ATGTTGCTGCTTATCATACATATCATCATTCTGATAGTGTTC ACTACCATCTACAAGATGCTCCCCGGTGGCATGTTCTCGAA CACGGACCCGACTTGGGTTGATTGCCTGTACTTTTCGGCAT CGACGCACACCACCGTGGGGTACGGAGATCTCACGCCCA AATCACCCGTGGCAAAACTCACGGCAACGGCACACATGT TGATCGTATTCGCGATCGTCATTTCCGGCTTCA CGTTCCCGTGGTAG	Platte River; Louisville, Nebraska	10/26/2017	K2	Can0610SP
KS3-S (1)	ATGTTGCTGCTTATCATACATATCATCATTCTGATAG TGTTCACTACCATCTACAAGATGCTCCCCGGCGGCATG TTCTCGAACACAGACCCTACTTGGGTCGATTGCCTGTA CTTTTCGGCATCGACGCACACCACCGTGGGGTACGGAG ATCTCACGCCCAAATCACCCGTGGCAAAACTCACGGCA ACGGCACACATGTTGATCGTATTCGCGATCGTCATTT CCGGCTTCACATTTCCGTGGTAA	Delaware River; Valley Fall, Kansas	Jun-17	KS3-S	Can0610SP

Table A1. *Cont.*

Sample ID	DNA Sequence	Location collected	Date collected	DNA Group	Amino Acid Group
LP_CO_F1m (7)	ATGTTGCTGCTTATCATACATATCATCATTCTGATAG TGTTCACTACCATCTACAAGATGCTCCCCGGTGGCA TGTTCTCGAACACGGACCCGACTTGGGTTGATTGCC TGTACTTTTCGGCATCGACGCACACCACCGTGGGGT ACGGAGATCTCACGCCCAAATCACCCGTGGCAAAA CTCACGGCAACGGCACACATGTTGATCGTATTCGCGA TCGTCATTTCCGGCTTCACGTTTCCGTGGTAA	Cache la Poudre River, Colorado	5/29/2017	LP_CO_F1m	Can0610SP
LP_CO_F2L	ATGTTGCTGCTTATCATACATATCATCATTCTGATA GTGTTCACTACCATCTACAAGATGCTCCCCGGTGGCAT GTTCTCGAACACGGACCCGACTTGGGTTGATTGCCTGT ACTTTTCGGCATCGACGCACACCACCGTGGGGTACGGA GATCTCACGCCCAAATCACCCGTGGCAAAACTCACGGC AACGGCACACATGTTGATCGTATTCGCGATCGTCATTTC CGGCTTCACGTTTCCGTGGTAA	Cache la Poudre River, Colorado	5/30/2017	LP_CO_F1m	Can0610SP
LP_CO_F2m	ATGTTGCTGCTTATCATACATATCATCATTCTGATAGTGTT CACTACCATCTACAAGATGCTCCCCGGTGGCATGTTCTCGA ACACGGACCCGACTTGGGTTGATTGCCTGTACTTTTCGGCA TCGACGCACACCACCGTGGGGTACGGAGATCTCACGCCCA AATCACCCGTGGCAAAACTCACGGCAACGGCACACATGTTG ATCGTATTCGCGATCGTCATTTCCGGCTTCACGTTTCCGTGGTAA	Cache la Poudre River, Colorado	5/31/2017	LP_CO_F1m	Can0610SP
LP_CO_F2s	ATGTTGCTGCTTATCATACATATCATCATTCTGATAGTGTT CACTACCATCTACAAGATGCTCCCCGGTGGCATGTTCTC GAACACGGACCCGACTTGGGTTGATTGCCTGTACTTTT CGGCATCGACGCACACCACCGTGGGGTACGGAGATCT CACGCCCAAATCACCCGTGGCAAAACTCACGGCAAC GGCACACATGTTGATCGTATTCGCGATC GTCATTTCCGGCTTCACGTTTCCGTGGTAA	Cache la Poudre River, Colorado	6/1/2017	LP_CO_F1m	Can0610SP
LP_CO_F3L	ATGTTGCTGCTTATCATACATATCATCATTCTGATAGTGTT CACTACCATCTACAAGATGCTCCCCGGTGGCATGTTCTC GAACACGGACCCGACTTGGGTTGATTGCCTGTACTTTT CGGCATCGACGCACACCACCGTGGGGTACGGAGATCT CACGCCCAAATCACCCGTGGCAAAACTCACGGCAAC GGCACACATGTTGATCGTATTCGCGATCGTCATTTC CGGCTTCACGTTTCCGTGGTAA	Cache la Poudre River, Colorado	6/2/2017	LP_CO_F1m	Can0610SP

Table A1. *Cont.*

Sample ID	DNA Sequence	Location collected	Date collected	DNA Group	Amino Acid Group
LP_CO_F3m	ATGTTGCTGCTTATCATACATATCATCATTCTGATAGTGTTCA CTACCATCTACAAGATGCTCCCCGGTGGCATGTTCTCGAAC ACGGACCCGACTTGGGTTGATTGCCTGTACTTTTCGGCATC GACGCACACCACCGTGGGGTACGGAGATCTCACGCCCAAA TCACCCGTGGCAAAACTCACGGCAACGGCACACATGTTG ATCGTATTCGCGATCGTCATTTCCGGCTTCACGTTTCC GTGGTAA	Cache la Poudre River, Colorado	6/3/2017	LP_CO_F1m	Can0610SP
LP_CO_F4s	ATGTTGCTGCTTATCATACATATCATCATTCTGATAGTGT TCACTACCATCTACAAGATGCTCCCCGGTGGCATGTTCTC GAACACGGACCCGACTTGGGTTGATTGCCTGTACTTTTCG GCATCGACGCACACCACCGTGGGGTACGGAGATCTCACG CCCAAATCACCCGTGGCAAAACTCACGGCAACGGCACA CATGTTGATCGTATTCGCGATCGTCATTTC CGGCTTCACGTTTCCGTGGTAA	Cache la Poudre River, Colorado	6/4/2017	LP_CO_F1m	Can0610SP
WR_DE (2)	ATGTTGCTGCTTATCATACATATCATCATTCTGATA GTGTTCACTACCATATACAAGATGCTCCCCGGCGGC ATGTTCTCGAACACAGACCCTACTTGGGTCGATTGC CTGTACTTTTCGGCATCGACGCACACCACCGTGGGG TACGGAGATCTCACGCCCAAATCACCCGTGGCAAAA CTCACGGCAACGGCGCACATGTTGATCGTATTCGCG ATCGTCATTTCTGGATTCACGTTCCCGTGGTAG	Wilson Run: Winterhur, Delaware	Jul-17	WR_DE	Can0610SP
WR_DE_s2cr2	ATGTTGCTGCTTATCATACATATCATCATTCTGATAGT GTTCACTACCATATACAAGATGCTCCCCGGCGGCAT GTTCTCGAACACAGACCCTACTTGGGTCGATTGCC TGTACTTTTCGGCATCGACGCACACCACCGTGGGG TACGGAGATCTCACGCCCAAATCACCCGTGGCAAA ACTCACGGCAACGGCGCACATGTTGATCGTATTCG CGATCG TCATTTCTGGATTCACGTTCCCGTGGTAG	Wilson Run, Winterthur; Delaware	Jul-17	WR_DE	Can0610SP
Canal1 (2) [2]	ATGTTGCTGCTCCTTATACACGTTGGTATTTTGGTATTTT TCACCACCGTATACAAGATGCTCCCCGGTGGCATGTTC TCGAATACGGACCCTAGCTGGGTAGATTGCTTATACTTC TCAGCGTCAACTCACACCACCGTTGGGTACGGAGATC TCACGCCCAAATCACCCGTGGCGAAACTCGTGGCGAC GGCGCATATGATGATCGTGTTCGCGATCGTTGTATCTAG CTTCACGTTTTCGTGGTAG	Canal exiting Smith Lake, Nebraska	2008	Canal1	Canal1

Table A1. *Cont.*

Sample ID	DNA Sequence	Location collected	Date collected	DNA Group	Amino Acid Group
Smith-Lake_ Small-Plaque	ATGTTGCTGCTCCTTATACACGTTGGTATTTTGGTATTTT TCACCACCGTATACAAGATGCTCCCCGGTGGCATGTTCTCGAA TACGGACCCTAGCTGGGTAGATTGCTTATACTTCTCAGCGT CAACTCACACCACCGTTGGGTACGGAGATCTCACGCCCAA ATCACCCGTGGCGAAACTCGTGGCGACGGCGCATATGAT GATCGTGTTCGCGATCGTTGTATCTAGCT TCACGTTTTCGTGGTAG	Smith Lake, Western Nebraska	2018	Canal1	Canal1
GM0701 (1) [1]	ATGTTGCTGCTTCTCATACATCTCAGCATTTTGGTACTTTT CACTACCATATACAAGATGCTCCCCGGCGGCATGTTC TCGAACACGGACCCATCCTGGGTCGATTGCCTGTAC TTTTCAGCATCAACGCACACGACCGTGGGGTACGG GGACCTCACGCCAAAATCGCCCGTGGCAAAACTC ACAGCAACGGCACACATGCTGATCGTATTC GCGATCGTAATAACTGGCTTCACATT CCCGTGGTAA	Guatemala	2007	GM0701	GM0701
GNLD22 (1) [1]	ATGCTGCGGTCAATATTGCCTCATATCATAGTGTTCAC GTTTTTCGTTGTTCTTTACAAATTTTTCCCCGGGGGG TTTGAAGACTCATTCAAACGAGGAGACGGGTCCCGCA GAAAGGCGACGTGGATGGACTGCATCTATTTCGCGACG GCAACGCACACCACCACCGGGTTTGGTGATGTAGTCC CCGACAACGACGCCGCAAGAACAGCTGTCACGATGC ACATGCTCATAGTTTTCGCGATCGTAGTATT GGGGATAAAACTCTAA	Lake Sisimiut, Greenland	2012	GNLD22	GNLD22
Hale-L (2) [6]	ATGTTGCTGCTCCTTATACACATCGGTATTTTGGTATTT TTCACTATCGTGTACAAGCTGCTCCCTGGTGGCATGTT CTCGTACGCAGACCCGACCTGGGTCGACTGCTTGTATT TTTCGGCATCAACGCACACCACCGTGGGGTATGGGGAT CTCACGCCCAAATCACCCGTGGCAAAACTCACGGCC ACGGCACACATGCTGATTGTATTCGCGATCGTTGTCTC TAGCTTTACGCTCCCCTGGTAA	North branch of Elk Creek; Hale, Wisconsin	7/4/2017	Hale-L	Hale-L
Hale_WI_m4	ATGTTGCTGCTCCTTATACACATCGGTATTTTGGTATTTTT CACTATCGTGTACAAGCTGCTCCCTGGTGGCATGTTCTCGT ACGCAGACCCGACCTGGGTCGACTGCTTGTATTTTTCGGCA TCAACGCACACCACCGTGGGGTATGGGGATCTCACGCCCA AATCACCCGTGGCAAAACTCACGGCCACGGCACACATGCT GATTGTATTCGCGATCGTTGTCTCTAGCTTTAC GCTCCCCTGGTAA	North branch of Elk Creek; Hale, Wisconsin	7/4/2017	Hale-L	Hale-L

Table A1. *Cont.*

Sample ID	DNA Sequence	Location collected	Date collected	DNA Group	Amino Acid Group
TX3-L1 (4)	ATGTTGCTGCTCCTTATACACATTGGTATTTTGGTATTTTTC ACTATCGTGTACAAACTGCTCCCTGGTGGCATGTTCTCGT ACGCAGATCCGACCTGGGTCGACTGCTTGTATTTTTCGGC ATCAACGCACACCACCGTGGGGTATGGGGATCTCACGCC CAAATCACCCGTGGCAAAACTCACGGCCACTGCACACA TGCTGATTGTATTCGCGATCGTTGTCTCTAGCTTTAC GCTCCCCTGGTAA	Colorado River Pond; Austin, Texas	Jul-17	TX3-L1	Hale-L
TX3-L2	ATGTTGCTGCTCCTTATACACATTGGTATTTTGGTATTTTT CACTATCGTGTACAAACTGCTCCCTGGTGGCATGTTCTC GTACGCAGATCCGACCTGGGTCGACTGCTTGTATTTTTCG GCATCAACGCACACCACCGTGGGGTATGGGGATCTCACG CCCAAATCACCCGTGGCAAAACTCACGGCCACTGCACAC ATGCTGATTGTATTCGCGATCGTTGTCTCTAGCTTTA CGCTCCCCTGGTAA	Colorado River Pond, Austin, Texas	Jul-17	TX3-L1	Hale-L
TX3m	ATGTTGCTGCTCCTTATACACATTGGTATTTTGGTATTT TTCACTATCGTGTACAAACTGCTCCCTGGTGGCATGTTC TCGTACGCAGATCCGACCTGGGTCGACTGCTTGTATTTT TCGGCATCAACGCACACCACCGTGGGGTATGGGGATCTC ACGCCCAAATCACCCGTGGCAAAACTCACGGCCACTGC ACACATGCTGATTGTATTCGCGATCGTTGTCTCTAGCTTT ACGCTCCCCTGGTAA	Colorado River Pond; Austin, Texas	Jul-17	TX3-L1	Hale-L
TX3s	ATGTTGCTGCTCCTTATACACATTGGTATTTTGGTATTTTT CACTATCGTGTACAAACTGCTCCCTGGTGGCATGTTCTC GTACGCAGATCCGACCTGGGTCGACTGCTTGTATTTTTC GGCATCAACGCACACCACCGTGGGGTATGGGGATCTCA CGCCCAAATCACCCGTGGCAAAACTCACGGCCACTGC ACACATGCTGATTGTATTCGCGATCGTTGTCTCTAGCTTT ACGCTCCCCTGGTAA	Colorado River Pond; Austin, Texas	Jul-17	TX3-L1	Hale-L
Hale-s1 (2) [2]	ATGTTGCTGCTCCTTATACACATCGGTATTTTGGTATTTTT CACTATCGTGTACAAGCTGCTCCCTGGTGGCATGTTCTCG TACGCAGACCCGACCTGGGTCGACTGCTTGTATTTTTCGG CATCAACGCACACCACCGTGGGGTATGGGGATCTCACGC CCAAATCACCCGTGGCAAAACTCACGGCAACGGCACAC ATGCTGATCGTACTCGCGATCGTCATTTCTGGCTTCACGT TCCCGTGGTAG	North branch of Elk Creek; Hale, Wisconsin	7/4/2017	Hale-s1	Hale-s1

Table A1. *Cont.*

Sample ID	DNA Sequence	Location collected	Date collected	DNA Group	Amino Acid Group
Hale-s2	ATGTTGCTGCTCCTTATACACATCGGTATTTTGGTATTTTTC ACTATCGTGTACAAGCTGCTCCCTGGTGGCATGTTCTCGT ACGCAGACCCGACCTGGGTCGACTGCTTGTATTTTTCGG CATCAACGCACACCACCGTGGGGTATGGGGATCTCACGC CCAAATCACCCGTGGCAAAACTCACGGCAACGGCACA CATGCTGATCGTACTCGCGATCGTCATTTCTGGCTTCAC GTTCCCGTGGTAG	North branch of Elk Creek; Hale, Wisconsin	7/4/2017	Hale-s1	Hale-s1
IL-1 (1) [6]	ATGTTGCTGCTTCTCATACATCTCAGCATTTTGGTAATT TTCACTACCATATACAAGATGCTCCCCGGCGGCATGT TCTCGAACACGGATCCGTCCTGGGTCGATTGCCTGTA CTTTTCGGCATCAACGCACACCACCGTGGGGTACGG GGACCTCACGCCAAAATCACCCGTGGCAAAACTCA CGGCAACGGCACACATGCTGATCGTATTTGCGATCGT CATTTCTGGCTTCACGTTCCCGTGGTAA	Lake Zurich, Illinois	6/27/2017	IL-1	IL-1
Dismal_NE (1)	ATGTTGCTGCTTCTCATACATCTCAGCATTTTGGTA ATTTTCACTACCATCTACAAGATGCTCCCCGGCGGC ATGTTCTCGAACACGGACCCATCCTGGGTCGATTGC CTGTACTTTTCGGCATCAACGCACACCACCGTGGG GTACGGGGACCTCACGCCAAAATCACCCGTGGCA AAACTCACGGCAACGGCACACATGCTGATCGTATT CGCGATCGTCATTTCTGGCTTCACGTTCCCGTGGTAG	Dismal River, Nebraska	5/26/2017	Dismal_NE	IL-1
NY2s1 (2)	ATGTTGCTGCTTCTCATACATCTCAGCATTTTGGTAATTTTCA CTACCATCTACAAGATGCTCCCCGGCGGCATGTTCTCGAAC ACGGACCCATCCTGGGTCGATTGCCTGTACTTTTCGGCAT CAACGCACACCACCGTGGGGTACGGGGACCTCACGCCCA AATCACCCGTTGCAAAACTCACGGCAACGGCACACATGC TGATCGTATTCGCGATCGTCATTTCTGGCTTCACGTT CCCGTGGTAA	Moodna Creek; Washingtonville, New York	6/21/2017	NY-2s1	IL-1
NY-2s2	ATGTTGCTGCTTCTCATACATCTCAGCATTTTGGTAAT TTTCACTACCATCTACAAGATGCTCCCCGGCGGCAT GTTCTCGAACACGGACCCATCCTGGGTCGATTGCCT GTACTTTTCGGCATCAACGCACACCACCGTGGGGT ACGGGGACCTCACGCCCAAATCACCCGTTGCAAAA CTCACGGCAACGGCACACATGCTGATCGTATTCGC GATCGTCATTTCTGGCTTCACGTTCCCGTGGTAA	Moodna Creek; Washingtonville, New York	6/21/2017	NY-2s1	IL-1

Table A1. *Cont.*

Sample ID	DNA Sequence	Location collected	Date collected	DNA Group	Amino Acid Group
Pl_R_OLa (1)	ATGTTGCTGCTTCTCATACATCTCAGCATTTTGGTA ATTTTCACTACCATCTACAAGATGCTCCCCGGCGG CATGTTCTCGAACACGGACCCATCCTGGGTCGAT TGCCTGTACTTTTCGGCATCAACGCACACCACCGT GGGGTACGGGGACCTCACGCCAAAATCACCCGTG GCAAAACTCACGGCAACGGCACACATGCTGATCG TATTCGCGATCGTCATTTCTGGCTTCACGTTCCCATGGTAG	Platte River; Odessa, Nebraska	10/23/2017	Pl_R_Ola	IL-1
Somers_MT_m1 (1)	ATGTTGCTGCTTCTCATACATCTCAGCATTTTGGTA ATTTTCACTACCATATACAAGATGCTCCCCGGCGGC ATGTTCTCAAACACGGATCCGTCCTGGGTCGATTGC CTGTACTTTTCGGCATCAACGCACACCACCGTGGG GTACGGGGACCTCACGCCAAAATCACCCGTGGCAA AACTCACGGCAACGGCACACATGCTGATCGTATTC GCGATCGTCATTTCTGGCTTCACGTTCCCGTGGTAA	Flathead Lake; Somers, Montana	6/27/2017	Somers_MT_m1	IL-1
IL-M (1) [1]	ATGTTGCTGCTTATCATACATCTCAGCATTTTGGTAA TTTTCACTACCATATACAAGATGCTCCCCGGCGGCA TGTTCTCGAACACGGATCCGTCCTGGGTCGATTGCC TGTACTTTTCGGCATCAACGCACACCACCGTGGGGT ACGGGGACCTCACGCCAAAATCACCCGTGGCAAAA CTCACGGCAACGGCACACATGCTGATCGTATTTGCG ATCGTCATTTCTGGCTTCACATTTCCGTGGTAG	Lake Zurich, Illinois	6/27/2017	IL-M	IL-M
Island-Lake_ Medium (1) [1]	ATGTTGCTGCTCCTTATCCACGTGTGTATTTTGACAGTC TTCACGATTGTTTACAAGATGCTCCCTGGCGGCATGTTC TCTAACGCGGACCCGTCGTGGGTAGACTGCTTATACTTT GCCGCGTCGACTCACACCACAGTGGGGTACGGGGACC TCACCCCCAAATCGCCAGTGGCAAAGCTCACGGCGAC GGCCCACATGTTGATCGTGTTCGCGATCATTATATC TAGCTTCACACTGCCATGGTAG	Island Lake, Western Nebraska	2018	Island-Lake_Medium	Island-Lake_Medium
Island-Lake_Small (1) [1]	ATGTTGCTGCTTATCATACATATCGTCATTCTTATAGTG TTCACTACCATCTACAAGATGCTCCCCGGCGGCATGTT CTCGAACACGGACCCGACTTGGGTTGATTGCCTGTACT TTTCGGCATCGACGCACACCACCGTGGGGTACGGAGA TCTCACGCCCAAATCACCCGTGGCAAAGCTCACGGCA ACGGCACACATGCTGATCGTATTCGCGATCGTCATTTCT GGCTTCACGTTTCCGTGGTAG	Island Lake, Western Nebraska	2018	Island-Lake_Small	Island-Lake_Small

Table A1. *Cont.*

Sample ID	DNA Sequence	Location collected	Date collected	DNA Group	Amino Acid Group
mid_1.1 (1) [20]	ATGAAGCTGCTACTTTCACATATTGTTATTCTAATATGTT TCACCGTCGTGTACAAGATGCTCCCCGGTGGCATGTTC TCGGAAGCAGACCCGTCGTGGGTTGACTGTCTGTATTT CTCGACGGCAACACACACGACAACGGGCTACGGCGAT CTAACGCCAGAAACCCCGGTGGCAAAACTCGTGACAA CGGTGCACATGTTAACCGTGTTCATCATCGTTATTTCCG GCTTCACTGGCTTCGCATTATGGTAG	Germany	Oct-17	mid_1.1	mid_1.1
up_1.2m1 (19)	ATGAAGCTGCTACTTTCACATATCGTTATTCTAATATGTTT CACCGTCGTGTACAAGATGCTCCCCGGTGGCATGTTCT CGGAAGCAGACCCGTCGTGGGTTGACTGTCTGTATTTC TCGACGGCAACACACACGACAACGGGCTACGGCGATC TAACGCCAGAAACCCCGGTGGCAAAACTCGTGACAAC GGTGCACATGTTAACCGTGTTCATCATCGTTATTTCCGG CTTCACTGGCTTCGCATTATGGTAG	Germany	Oct-17	up_1.2m1	mid_1.1
mid_1.2m	ATGAAGCTGCTACTTTCACATATCGTTATTCTAATATGTT TCACCGTCGTGTACAAGATGCTCCCCGGTGGCATGTTCT CGGAAGCAGACCCGTCGTGGGTTGACTGTCTGTATTTCT CGACGGCAACACACACGACAACGGGCTACGGCGATCTA ACGCCAGAAACCCCGGTGGCAAAACTCGTGACAACGG TGCACATGTTAACCGTGTTCATCATCGTTATTTCCGGCTTC ACTGGCTTCGCATTATGGTAG	Germany	Oct-17	up_1.2m1	mid_1.1
mid_1.2s2	ATGAAGCTGCTACTTTCACATATCGTTATTCTAATATGTTTC ACCGTCGTGTACAAGATGCTCCCCGGTGGCATGTTCTCGGA AGCAGACCCGTCGTGGGTTGACTGTCTGTATTTCTCGACG GCAACACACACGACAACGGGCTACGGCGATCTAACGCCA GAAACCCCGGTGGCAAAACTCGTGACAACGGTGCACATG TTAACCGTGTTCATCATCGTTATTTCCGGCTTCACTGGCTT CGCATTATGGTAG	Germany	Oct-17	up_1.2m1	mid_1.1
mid_10.1L	ATGAAGCTGCTACTTTCACATATCGTTATTCTAATATGTTTC ACCGTCGTGTACAAGATGCTCCCCGGTGGCATGTTCTCGG AAGCAGACCCGTCGTGGGTTGACTGTCTGTATTTCTCGAC GGCAACACACACGACAACGGGCTACGGCGATCTAACGCC AGAAACCCCGGTGGCAAAACTCGTGACAACGGTGCACAT GTTAACCGTGTTCATCATCGTTATT TCCGGCTTCACTGGCTTCGCATTATGGTAG	Germany	Oct-17	up_1.2m1	mid_1.1

Table A1. *Cont.*

Sample ID	DNA Sequence	Location collected	Date collected	DNA Group	Amino Acid Group
mid_10.1s1	ATGAAGCTGCTACTTTCACATATCGTTATTCTAATATGTTTC ACCGTCGTGTACAAGATGCTCCCCGGTGGCATGTTCTCGG AAGCAGACCCGTCGTGGGTTGACTGTCTGTATTTCTCGAC GGCAACACACACGACAACGGGCTACGGCGATCTAACGCC AGAAACCCCCGGTGGCAAAACTCGTGACAACGGTGCACA TGTTAACCGTGTTCATCATCGTTATTTCCGGCTTCACTGGC TTCGCATTATGGTAG	Germany	Oct-17	up_1.2m1	mid_1.1
mid_10.1s2	ATGAAGCTGCTACTTTCACATATCGTTATTCTAATATGTTTC ACCGTCGTGTACAAGATGCTCCCCGGTGGCATGTTCTCG GAAGCAGACCCGTCGTGGGTTGACTGTCTGTATTTCTCG ACGGCAACACACACGACAACGGGCTACGGCGATCTAAC GCCAGAAACCCCGGTGGCAAAACTCGTGACAACGGTG CACATGTTAACCGTGTTCATCATCGTTATTTCCGGCTTCA CTGGCTTCGCATTATGGTAG	Germany	Oct-17	up_1.2m1	mid_1.1
mid_11.1m	ATGAAGCTGCTACTTTCACATATCGTTATTCTAATATGTTT CACCGTCGTGTACAAGATGCTCCCCGGTGGCATGTTCTCGGA AGCAGACCCGTCGTGGGTTGACTGTCTGTATTTCTCGACGG CAACACACACGACAACGGGCTACGGCGATCTAACGCCAG AAACCCCGGTGGCAAAACTCGTGACAACGGTGCACATGT TAACCGTGTTCATCATCGTTATTTCCGGCTTCACTGGCTTCG CATTATGGTAG	Germany	Oct-17	up_1.2m1	mid_1.1
mid_13.1L1	ATGAAGCTGCTACTTTCACATATCGTTATTCTAATATGTTTC ACCGTCGTGTACAAGATGCTCCCCGGTGGCATGTTCTCGG AAGCAGACCCGTCGTGGGTTGACTGTCTGTATTTCTCGAC GGCAACACACACGACAACGGGCTACGGCGATCTAACGCC AGAAACCCCCGGTGGCAAAACTCGTGACAACGGTGCACA TGTTAACCGTGTTCATCATCGTTATTTCCGGCTTCACTGGC TTCGCATTATGGTAG	Germany	Oct-17	up_1.2m1	mid_1.1
mid_13.1L2	ATGAAGCTGCTACTTTCACATATCGTTATTCTAATATGTTT CACCGTCGTGTACAAGATGCTCCCCGGTGGCATGTTCTC GGAAGCAGACCCGTCGTGGGTTGACTGTCTGTATTTCTC GACGGCAACACACACGACAACGGGCTACGGCGATCTAA CGCCAGAAACCCCCGGTGGCAAAACTCGTGACAACGGT GCACATGTTAACCGTGTTCATCATCGTTATTTCCGGCTTC ACTGGCTTCGCATTATGGTAG	Germany	Oct-17	up_1.2m1	mid_1.1

Table A1. *Cont.*

Sample ID	DNA Sequence	Location collected	Date collected	DNA Group	Amino Acid Group
mid_13.1s1	ATGAAGCTGCTACTTTCACATATCGTTATTCTAATATGTTT CACCGTCGTGTACAAGATGCTCCCCGGTGGCATGTTCTC GGAAGCAGACCCGTCGTGGGTTGACTGTCTGTATTTCTC GACGGCAACACACACGACAACGGGCTACGGCGATCTAA CGCCAGAAACCCCGGTGGCAAAACTCGTGACAACGGTG CACATGTTAACCGTGTTCATCATCGTTATTTCCGGCTTCAC TGGCTTCGCATTATGGTAG	Germany	Oct-17	up_1.2m1	mid_1.1
mid_5.1L1	ATGAAGCTGCTACTTTCACATATCGTTATTCTAATATGTTTC ACCGTCGTGTACAAGATGCTCCCCGGTGGCATGTTCTCG GAAGCAGACCCGTCGTGGGTTGACTGTCTGTATTTCTCG ACGGCAACACACACGACAACGGGCTACGGCGATCTAA CGCCAGAAACCCCGGTGGCAAAACTCGTGACAACGGT GCACATGTTAACCGTGTTCATCATCGTTATTTCCGGCTTC ACTGGCTTCGCATTATGGTAG	Germany	Oct-17	up_1.2m1	mid_1.1
mid_5.1L2	ATGAAGCTGCTACTTTCACATATCGTTATTCTAATATGTT TCACCGTCGTGTACAAGATGCTCCCCGGTGGCATGTTCT CGGAAGCAGACCCGTCGTGGGTTGACTGTCTGTATTTCT CGACGGCAACACACACGACAACGGGCTACGGCGATCTA ACGCCAGAAACCCCGGTGGCAAAACTCGTGACAACGG TGCACATGTTAACCGTGTTCATCATCGTTATTTCCGGCTT CACTGGCTTCGCATTATGGTAG	Germany	Oct-17	up_1.2m1	mid_1.1
mid_5.1s1	ATGAAGCTGCTACTTTCACATATCGTTATTCTAATATGTTTC ACCGTCGTGTACAAGATGCTCCCCGGTGGCATGTTCTCGG AAGCAGACCCGTCGTGGGTTGACTGTCTGTATTTCTCGAC GGCAACACACACGACAACGGGCTACGGCGATCTAACGCC AGAAACCCCGGTGGCAAAACTCGTGACAACGGTGCACAT GTTAACCGTGTTCATCATCGTTATTTCCGGCTTCACTGGCT TCGCATTATGGTAG	Germany	Oct-17	up_1.2m1	mid_1.1
mid_7.2	ATGAAGCTGCTACTTTCACATATCGTTATTCTAATATGTTTCACCG TCGTGTACAAGATGCTCCCCGGTGGCATGTTCTCGGAAGCAGAC CCGTCGTGGGTTGACTGTCTGTATTTCTCGACGGCAACACACAC GACAACGGGCTACGGCGATCTAACGCCAGAAACCCCGGTGGC AAAACTCGTGACAACGGTGCACATGTTAACCGTGTTCATCATC GTTATTTCCGGCTTCACTGGCTTCGCATTATGGTAG	Germany	Oct-17	up_1.2m1	mid_1.1

Table A1. *Cont.*

Sample ID	DNA Sequence	Location collected	Date collected	DNA Group	Amino Acid Group
up_1.2m2	ATGAAGCTGCTACTTTCACATATCGTTATTCTAATATGTTTCAC CGTCGTGTACAAGATGCTCCCCGGTGGCATGTTCTCGGAAGC AGACCCGTCGTGGGTTGACTGTCTGTATTTCTCGACGGCAAC ACACACGACAACGGGCTACGGCGATCTAACGCCAGAAACCC CGGTGGCAAAACTCGTGACAACGGTGCACATGTTAACCGTG TTCATCATCGTTATTTCCGGCTTCACTGGCTTCGCATTATGGTAG	Germany	Oct-17	up_1.2m1	mid_1.1
up_4.1L1	ATGAAGCTGCTACTTTCACATATCGTTATTCTAATATGTTTCAC CGTCGTGTACAAGATGCTCCCCGGTGGCATGTTCTCGGAAGC AGACCCGTCGTGGGTTGACTGTCTGTATTTCTCGACGGCAAC ACACACGACAACGGGCTACGGCGATCTAACGCCAGAAACCC CGGTGGCAAAACTCGTGACAACGGTGCACATGTTAACCGTG TTCATCATCGTTATTTCCGGCTTCACTGGCTTCGCATTATGGTAG	Germany	Oct-17	up_1.2m1	mid_1.1
up_4.2	ATGAAGCTGCTACTTTCACATATCGTTATTCTAATATGTTTCAC CGTCGTGTACAAGATGCTCCCCGGTGGCATGTTCTCGGAAG CAGACCCGTCGTGGGTTGACTGTCTGTATTTCTCGACGGCA ACACACACGACAACGGGCTACGGCGATCTAACGCCAGAAA CCCCGGTGGCAAAACTCGTGACAACGGTGCACATGTTAAC CGTGTTCATCATCGTTATTTCCGGCTTCACTGGCTTCGCATT ATGGTAG	Germany	Oct-17	up_1.2m1	mid_1.1
up_7.2	ATGAAGCTGCTACTTTCACATATCGTTATTCTAATATGTTTCA CCGTCGTGTACAAGATGCTCCCCGGTGGCATGTTCTCGGA AGCAGACCCGTCGTGGGTTGACTGTCTGTATTTCTCGACG GCAACACACACGACAACGGGCTACGGCGATCTAACGCCA GAAACCCCGGTGGCAAAACTCGTGACAACGGTGCACAT GTTAACCGTGTTCATCATCGTTATTTCCGGCTTCACTGG CTTCGCATTATGGTAG	Germany	Oct-17	up_1.2m1	mid_1.1
up_8.1	ATGAAGCTGCTACTTTCACATATCGTTATTCTAATATGTTTCA CCGTCGTGTACAAGATGCTCCCCGGTGGCATGTTCTCGGAA GCAGACCCGTCGTGGGTTGACTGTCTGTATTTCTCGACGGC AACACACACGACAACGGGCTACGGCGATCTAACGCCAGA AACCCCGGTGGCAAAACTCGTGACAACGGTGCACATGTT AACCGTGTTCATCATCGTTATTTCCGGCTTCACTGGCTTCG CATTATGGTAG	Germany	Oct-17	up_1.2m1	mid_1.1

Table A1. *Cont.*

Sample ID	DNA Sequence	Location collected	Date collected	DNA Group	Amino Acid Group
mid_3.1s (4) [4]	ATGAAGCTGCTACTTTCACATATCGTTATTCTAATATGTTTCA CCGTTATTTACAAGATGCTCCCCGGTGGCATGTTCTCGGAT GCGGACCCGTCGTGGTTTGACTGTCTGTATTTCTCGACGG CGACGCATACGACAACAGGCTACGGCGATCTAACGCCTAA GACGCCGGTGGCAAAACTCGTGACCACAGCGCATATGTTA ACCGTTTTCGCGATCGTTATTTCCGGTTTCGCTGGCTTCAA GTTATGGTAG	Germany	Oct-17	mid_3.1s	mid_3.1s
mid_14.1	ATGAAGCTGCTACTTTCACATATCGTTATTCTAATATGTTTC ACCGTTATTTACAAGATGCTCCCCGGTGGCATGTTCTCGGA TGCGGACCCGTCGTGGTTTGACTGTCTGTATTTCTCGACGG CGACGCATACGACAACAGGCTACGGCGATCTAACGCCTAA GACGCCGGTGGCAAAACTCGTGACCACAGCGCATATGTTA ACCGTTTTCGCGATCGTTATTTCCGGTTTCGCTGGCTTCAA GTTATGGTAG	Germany	Oct-17	mid_3.1s	mid_3.1s
mid_14.2	ATGAAGCTGCTACTTTCACATATCGTTATTCTAATATGTTTCA CCGTTATTTACAAGATGCTCCCCGGTGGCATGTTCTCGGATG CGGACCCGTCGTGGTTTGACTGTCTGTATTTCTCGACGGCGA CGCATACGACAACAGGCTACGGCGATCTAACGCCTAAGAC GCCGGTGGCAAAACTCGTGACCACAGCGCATATGTTAACC GTTTTCGCGATCGTTATTTCCGGTTTCGCTGGCTTCAAGTTA TGGTAG	Germany	Oct-17	mid_3.1s	mid_3.1s
mid_14.3	ATGAAGCTGCTACTTTCACATATCGTTATTCTAATATGTTTC ACCGTTATTTACAAGATGCTCCCCGGTGGCATGTTCTCGGA TGCGGACCCGTCGTGGTTTGACTGTCTGTATTTCTCGACGG CGACGCATACGACAACAGGCTACGGCGATCTAACGCCTA AGACGCCGGTGGCAAAACTCGTGACCACAGCGCATATG TTAACCGTTTTCGCGATCGTTATTTCCGGTTTCGCTGGC TTCAAGTTATGGTAG	Germany	Oct-17	mid_3.1s	mid_3.1s
mid_6.1 (1) [1]	ATGAAGCTGCTACTTTCACATATCGTTATTCTAATATGTTTC ACCGTTATTTACAAGATGCTCCCCGGCGGCATGTTCTCGG ATGCAGACCCGTCGTGGTTTGACTGTCTGTATTTCTCGAC GGCGACGCATACGACAACAGGCTACGGCGATCTAACGC CCAAGTCGCCGGTGGCAAAACTCGTTACCACGGTGCAT ATGTTAACCGTGTTCGCGATCGTTATTTCCGGGTTCGCTG GCTTCAAGNTTCCATGGTAG	Germany	Oct-17	mid_6.1	mid_6.1

Table A1. *Cont.*

Sample ID	DNA Sequence	Location collected	Date collected	DNA Group	Amino Acid Group
mid_6.2 (5) [5]	ATGAAGCTGCTACTTTCACATATCGTTATTCTAATATGTTTC ACCGTTATTTACAAGATGCTCCCCGGCGGCATGTTCTCGG ATGCAGACCCGTCGTGGTTTGACTGTCTGTATTTCTCGAC GGCGACGCATACGACAACAGGCTACGGCGATCTAACGCC CAAGTCGCCGGTGGCAAAACTCGTTACCACGGTGCATAT GTTAACCGTGTTCGCGATCGTTATTTCCGGGTTCGCTGGC TTCAAGTTTCCATGGTAG	Germany	Oct-17	mid_6.2	mid_6.2
mid_12.1	ATGAAGCTGCTACTTTCACATATCGTTATTCTAATATGTTT CACCGTTATTTACAAGATGCTCCCCGGCGGCATGTTCTC GGATGCAGACCCGTCGTGGTTTGACTGTCTGTATTTCTC GACGGCGACGCATACGACAACAGGCTACGGCGATCTA ACGCCCAAGTCGCCGGTGGCAAAACTCGTTACCACG GTGCATATGTTAACCGTGTTCGCGATCGTTATTTCCGG GTTCGCTGGCTTCAAGTTTCCATGGTAG	Germany	Oct-17	mid_6.2	mid_6.2
mid_12.3L	ATGAAGCTGCTACTTTCACATATCGTTATTCTAATAT GTTTCACCGTTATTTACAAGATGCTCCCCGGCGGCA TGTTCTCGGATGCAGACCCGTCGTGGTTTGACTGT CTGTATTTCTCGACGGCGACGCATACGACAACAGG CTACGGCGATCTAACGCCCAAGTCGCCGGTGGCAA AACTCGTTACCACGGTGCATATGTTAACCGTGTTCG CGATCGTTATTTCCGGGTTCGCTGGCTTCAAGTTT CCATGGTAG	Germany	Oct-17	mid_6.2	mid_6.2
mid_12.3s	ATGAAGCTGCTACTTTCACATATCGTTATTCTAATATG TTTCACCGTTATTTACAAGATGCTCCCCGGCGGCAT GTTCTCGGATGCAGACCCGTCGTGGTTTGACTGTCT GTATTTCTCGACGGCGACGCATACGACAACAGGCT ACGGCGATCTAACGCCCAAGTCGCCGGTGGCAAA ACTCGTTACCACGGTGCATATGTTAACCGTGTTCG CGATCGTTATTTCCGGGTTCGCTGGCTTCAAGTTT CCATGGTAG	Germany	Oct-17	mid_6.2	mid_6.2

Table A1. *Cont.*

Sample ID	DNA Sequence	Location collected	Date collected	DNA Group	Amino Acid Group
mid_8.1	ATGAAGCTGCTACTTTCACATATCGTTATTCTAATAT GTTTCACCGTTATTTACAAGATGCTCCCCGGCGGC ATGTTCTCGGATGCAGACCCGTCGTGGTTTGACTG TCTGTATTTCTCGACGGCGACGCATACGACAACA GGCTACGGCGATCTAACGCCCAAGTCGCCGGTGG CAAAACTCGTTACCACGGTGCATATGTTAACCGTG TTCGCGATCGTTATTTCCGGGTTCGCTGGCTTCAA GTTTCCATGGTAG	Germany	Oct-17	mid_6.2	mid_6.2
mid_9.1 (1) [1]	ATGAAGCTGCTACTTTCACATATCGTTATTCTAAT ATGTTTCACCGTTATTTACAAGATGCTCCCCGGTG GCATGTTCTCGGATGCGGACCCGTCGTGGTTTGA CTGTCTGTATTTCTCGACGGCGACGCATACGACA ACAGGCTACGGCGATCTAACGCCTAAGTCGCCG GTGGCAAAACTCGTGACCACGGTGCATATGTTA ACCGTTTTCGCGATCGTTATTTCCGGGTTCGCTG GCTTCAAGTTATGGTAG	Germany	Oct-17	mid_9.1	mid_9.1
MN08101 (1) [1]	ATGCTGCTTCTCCTGATACACATTGCCATATTGACATTC TTTACGGTCGTGTACAAGATGCTCCCCGACGGCGTG TTCTCGAACGGGGACCCGTCGTGGGTAGACTGCTTAT ACTTTTCCGCGTCCACTCACACCACCGTGGGATACGG GGACCTCACCCCCAAATCACCCGTGGCAAAACTCAC GGCAACGGCCCATATGATGATCGTGTTCGCGATTGTAG TGTCTAGCTTCACGTTCCCGTGGTAG	Minnesota	2008	MN08101	MN08101
NEJV2 (2) [6]	ATGTTGCTGCTTATCATACATATCATCATTCTGATAGTGT TCACTACCATCTACAAGATGCTCCCCGGTGGTATGTTC TCGAACACGGACCCGACTTGGGTTGATTGCCTGTACT TTTCGGCATCGACGCACACCACTGTGGGGTACGGAGA TCTCACGCCCAAATCACCCGTGGCAAAACTCACGGC AACGGCACACATGTTGATCGTATTCGCGATCGTCATTT CCGGCTTCACGTTTTCGTGGTAG	Rowe Bird Sanctuary; Gibbon, Nebraska	2008	NEJV2	NEJV2
Dismal_NE_s3	ATGTTGCTGCTTATCATACATATCATCATTCTGATAGTG TTCACTACCATCTACAAGATGCTCCCCGGTGGTATGTTCTCGAA CACGGACCCGACTTGGGTTGATTGCCTGTACTTTTCGGCATCGA CGCACACCACTGTGGGGTACGGAGATCTCACGCCCAAATCAC CCGTGGCAAAACTCACGGCAACGGCACACATGTTGATCGTAT TCGCGATCGTCATTTCCGGCTTCACGTTTTCGTGGTAG	Dismal River, Nebraska	5/26/2017	NEJV2	NEJV2

Table A1. *Cont.*

Sample ID	DNA Sequence	Location collected	Date collected	DNA Group	Amino Acid Group
NEJV3 (1)	ATGTTGCTGCTTATCATACATATCATCATTCTGATAGTGTTCACT ACCATCTACAAGATGCTCCCCGGCGGCATGTTCTCGAACACA GACCCGACTTGGGTTGATTGCCTGTACTTTTCGGCATCGACG CACACCACTGTGGGGTACGGAGATCTCACGCCCAAATCACC CGTGGCAAAACTCACGGCAACGGCACACATGTTGATCGTAT TCGCGATCGTCATTTCCGGCTTCACGTTTTCGTGGTAG	Gudmundsen Ranch, NE	2008	NEJV3	NEJV2
MO3 (3)	ATGTTGCTGCTTATCATACATATCATCATTCTGATAGTGTTCAC TACCATCTACAAGATGCTCCCCGGCGGCATGTTCTCGAACA CAGACCCTACTTGGGTTGATTGCCTGTACTTTTCGGCATCGA CGCACACCACCGTGGGGTACGGAGATCTCACGCCCAAATC ACCCGTGGCAAAACTCACGGCAACGGCACACATGTTGATC GTATTCGCGATCGTCATTTCCGGCTTCACGTTTTCGTGGTAG	Lake Lotawana, Missouri	5/31/2017	MO3	NEJV2
NY2m	ATGTTGCTGCTTATCATACATATCATCATTCTGATAGTGTTCA CTACCATCTACAAGATGCTCCCCGGCGGCATGTTCTCGAA CACAGACCCTACTTGGGTTGATTGCCTGTACTTTTCGGCA TCGACGCACACCACCGTGGGGTACGGAGATCTCACGCC CAAATCACCCGTGGCAAAACTCACGGCAACGGCACACA TGTTGATCGTATTCGCGATCGTCATTTCCGGCTTCACGTT TTCGTGGTAG	Moodna Creek; Washingtonville, New York	6/21/2017	MO3	NEJV2
Verbena_VA_s3	ATGTTGCTGCTTATCATACATATCATCATTCTGATAGTGT TCACTACCATCTACAAGATGCTCCCCGGCGGCATGTTC TCGAACACAGACCCTACTTGGGTTGATTGCCTGTACTTT TCGGCATCGACGCACACCACCGTGGGGTACGGAGATCT CACGCCCAAATCACCCGTGGCAAAACTCACGGCAACG GCACACATGTTGATCGTATTCGCGATCGTCATTTCCGGC TTCACGTTTTCGTGGTAG	South fork of the Shenandoah River; Verbena, Virginia	7/1/2017	MO3	NEJV2
NTS1 (1) [1]	ATGTTGCTGCTTCTCATACATCTCAGCATTTTGGTAATTTT CACTGCCATCTACAAGATGCTGCCCGGCGGCATGTTCTC AAACACAGACCCGACTTGGGTCGATTGCCTGTACTTTTC GGCATCAACGCACACCACCGTGGGGTACGGGGACCTC ACGCCAAAATCACCCGTGGCAAAACTCACGGCAACGG CACACATGCTGATCGTATTCGCGATCGTCATTTCTGGCTT CACGTTCCCGTGGTAG	Next to Smith Lake, Western Nebraska	2008	NTS1	NTS1

Table A1. *Cont.*

Sample ID	DNA Sequence	Location collected	Date collected	DNA Group	Amino Acid Group
NY-2 (1) [4]	ATGTTGCTGCTTCTCATACATCTCAGCATTTTGGTAATTTTC ACTACCATCTACAAGATGCTTCCCGGCGGCATGTTCTCGAA CACGGACCCATCCTGGGTCGATTGCCTGTACTTTTCGGCAT CAACACACACCACCGTGGGGTACGGGGACCTCACGCCAA AATCACCCGTGGCAAAACTCACGGCAACGGCACACATGC TGATCGTTTTCGCGATCGTCATTTCTGGCTTCACGTT CCCGTGGTAG	Moodna Creek; Washingtonville, New York	6/21/2017	NY-2	NY-2
Somers_MT_L3 (1)	ATGTTGCTGCTTCTCATACATCTCAGCATTTTGGTAATTTTC ACTACCATCTACAAGATGCTCCCCGGCGGCATGTTCTCGA ACACGGACCCATCCTGGGTCGATTGCCTGTACTTTTCGGC ATCAACGCACACCACCGTGGGGTACGGGGACCTCACGCC AAAATCACCCGTGGCAAAACTCACGGCAACGGCACACA TGCTGATCGTATTCGCGATCGTCATTTCTGGCTTCACGT TCCCGTGGTAA	Flathead Lake; Somers, Montana	6/27/2017	Somers_MT_L3	NY-2
Verbena_VA_L4 (2)	ATGTTGCTGCTTCTCATACATCTCAGCATTTTGGTAATTTT CACTACCATCTACAAGATGCTTCCCGGCGGCATGTTCTCG AACACGGACCCATCCTGGGTCGATTGCCTGTACTTTTCG GCATCAACGCACACCACCGTGGGGTACGGGGACCTCAC GCCAAAATCACCCGTGGCAAAACTCACGGCAACGGCA CACATGCTGATCGTTTTCGCGATCGTCATTTCTGGCTTC ACGTTCCCGTGGTAG	South fork of the Shenandoah River; Verbena, Virginia	7/1/2017	Verbena_VA_L4	NY-2
Verbena_VA_m1	ATGTTGCTGCTTCTCATACATCTCAGCATTTTGGTAATTTT CACTACCATCTACAAGATGCTTCCCGGCGGCATGTTCTC GAACACGGACCCATCCTGGGTCGATTGCCTGTACTTTTC GGCATCAACGCACACCACCGTGGGGTACGGGGACCTC ACGCCAAAATCACCCGTGGCAAAACTCACGGCAACGG CACACATGCTGATCGTTTTCGCGATCGTCATTTCTGGCT TCACGTTCCCGTGGTAG	South fork of the Shenandoah River; Verbena, Virginia	7/1/2017	Verbena_VA_L4	NY-2
Smith_L_La (3) [3]	ATGTTGCTGCTCCTTATCCACATGTGTATTTTGACATTCTT CACAGTTGTTTACAAGATGCTCCCTGGCGGCATGTTCTC TAACGCGGACCCGTCGTGGGTAGACTGCTTATACTTTGC CGCGTCGACTCACACCACGGTGGGGTACGGGGACCTCA CCCCCAAATCGCCAGTGGCAAAGCTCACGGCGACGGC CCACATGTTGATCGTGTTCGCGATCATTATATCTAGCTTC ACACTCCCATGGTAG	Smith Lake, Western Nebraska	10/22/2017	Smith_L_La	Smith_L_La

Table A1. *Cont.*

Sample ID	DNA Sequence	Location collected	Date collected	DNA Group	Amino Acid Group
Smith-Lake_ Medium-Plaque	ATGTTGCTGCTCCTTATCCACATGTGTATTTTGACATTCT TCACAGTTGTTTACAAGATGCTCCCTGGCGGCATGTTC TCTAACGCGGACCCGTCGTGGGTAGACTGCTTATACTT TGCCGCGTCGACTCACACCACGGTGGGGTACGGGGAC CTCACCCCCAAATCGCCAGTGGCAAAGCTCACGGCGA CGGCCCACATGTTGATCGTGTTCGCGATCATTATATCTA GCTTCACACTCCCATGGTAG	Smith Lake, Western Nebraska	2018	Smith_L_La	Smith_L_La
Island-Lake_ Large-Plaque	ATGTTGCTGCTCCTTATCCACATGTGTATTTTGACATTCTTC ACAGTTGTTTACAAGATGCTCCCTGGCGGCATGTTCTCTA ACGCGGACCCGTCGTGGGTAGACTGCTTATACTTTGCCGC GTCGACTCACACCACGGTGGGGTACGGGGACCTCACCC CCAAATCGCCAGTGGCAAAGCTCACGGCGACGGCCCAC ATGTTGATCGTGTTCGCGATCATTATATCTAGCTTCACACT CCCATGGTAG	Island Lake, Western Nebraska	2018	Smith_L_La	Smith_L_La
SPRLa (1) [1]	ATGTTGCTGCTCCTTATACACATCGGTATTTTGGTATTTTTC ACTATCGTGTACAAGATGCTCCCCGGCGGCATGTTCTCGA ACACAGACCCTACTTGGGTCGATTGCCTGTACTTTTCGGC ATCGACGCACACCACCGTGGGGTACGGAGATCTCACGCC CAAATCACCCGTGGCAAAACTCACGGCAACGGCACACAT GTTGATCGTATTCGCGATCGTCATTTCCGGCTTCACGTTTC CGTGGTAG	South Platte River; Big Spring, Nebraska	10/23/2017	SPRLa	SPRLa
TN603 (1) [1]	ATGTTGCTGCTTCTCATACACCTCTGTATTTTGATAATTTTTA CTACAATATACAAGATGTTGCCCGGAGGCATGTTCTCGAAC ACGGACCCGTCATGGATAGATTGCCTGTACTTCTCGGCATC AACGCACACCACCGTGGGGTACGGGGATCTCACGCCCAAA TCGCCCGTGGCAAAACTCACAGCAACGGCACACATGCTG ATCGTATTCGCGATCGTAATAACTGGCTTCACATTCCCG TGGTAA	Tennessee	2006	TN603	TN603
up_3.1 (4) [4]	ATGAAGCTGCTACTTTCACATATCGTTATTCTAATATGTTT CACCGTTATTTACAAGATGCTCCCCGGTGGCATGTTCTCG GATGCGGACCCGTCGTGGTTTGACTGTCTGTATTTCTCGA CGGCGACGCATACGACAACAGGCTACGGCGATCTAACG CCTAAGTCGCCGGTGGCAAAACTCGTGACCACAGCGCA TATGTTAACCGTTTTCGCGATCGTTATTTCCGGTTTCGCTG GCTTCAAGTTATGGTAG	Germany	Oct-17	up_3.1	up_3.1

Table A1. *Cont.*

Sample ID	DNA Sequence	Location collected	Date collected	DNA Group	Amino Acid Group
up_5.2L	ATGAAGCTGCTACTTTCACATATCGTTATTCTAATATGTTTC ACCGTTATTTACAAGATGCTCCCCGGTGGCATGTTCTCGG ATGCGGACCCGTCGTGGTTTGACTGTCTGTATTTCTCGAC GGCGACGCATACGACAACAGGCTACGGCGATCTAACG CCTAAGTCGCCGGTGGCAAAACTCGTGACCACAGCGC ATATGTTAACCGTTTTCGCGATCGTTATTTCCGGTTTCGC TGGCTTCAAGTTATGGTAG	Germany	Oct-17	up_3.1	up_3.1
up_5.3m	ATGAAGCTGCTACTTTCACATATCGTTATTCTAATATGTTT CACCGTTATTTACAAGATGCTCCCCGGTGGCATGTTCTC GGATGCGGACCCGTCGTGGTTTGACTGTCTGTATTTCTC GACGGCGACGCATACGACAACAGGCTACGGCGATCTA ACGCCTAAGTCGCCGGTGGCAAAACTCGTGACCACAG CGCATATGTTAACCGTTTTCGCGATCGTTATTTCCGGTT TCGCTGGCTTCAAGTTATGGTAG	Germany	Oct-17	up_3.1	up_3.1
up_5.3s2	ATGAAGCTGCTACTTTCACATATCGTTATTCTAATATGTTT CACCGTTATTTACAAGATGCTCCCCGGTGGCATGTTCTC GGATGCGGACCCGTCGTGGTTTGACTGTCTGTATTTCTC GACGGCGACGCATACGACAACAGGCTACGGCGATCTAA CGCCTAAGTCGCCGGTGGCAAAACTCGTGACCACAGC GCATATGTTAACCGTTTTCGCGATCGTTATTTCCGGTTTC GCTGGCTTCAAGTTATGGTAG	Germany	Oct-17	up_3.1	up_3.1

[1] Sequences submitted to the GenBank. The accession numbers for the *kcv* sequences in GenBank are MT560092–MT560194.

Appendix B

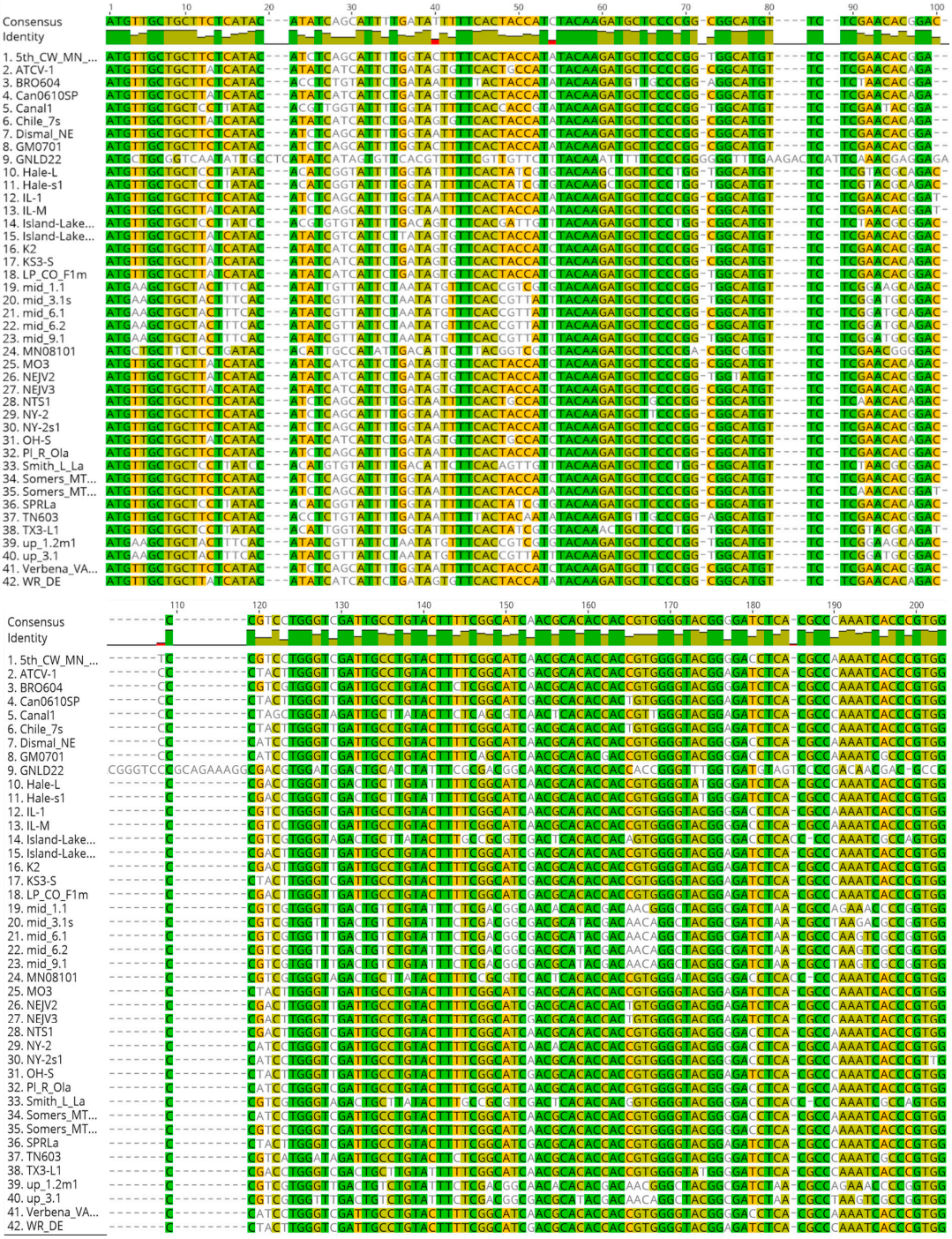

Figure A1. *Cont.*

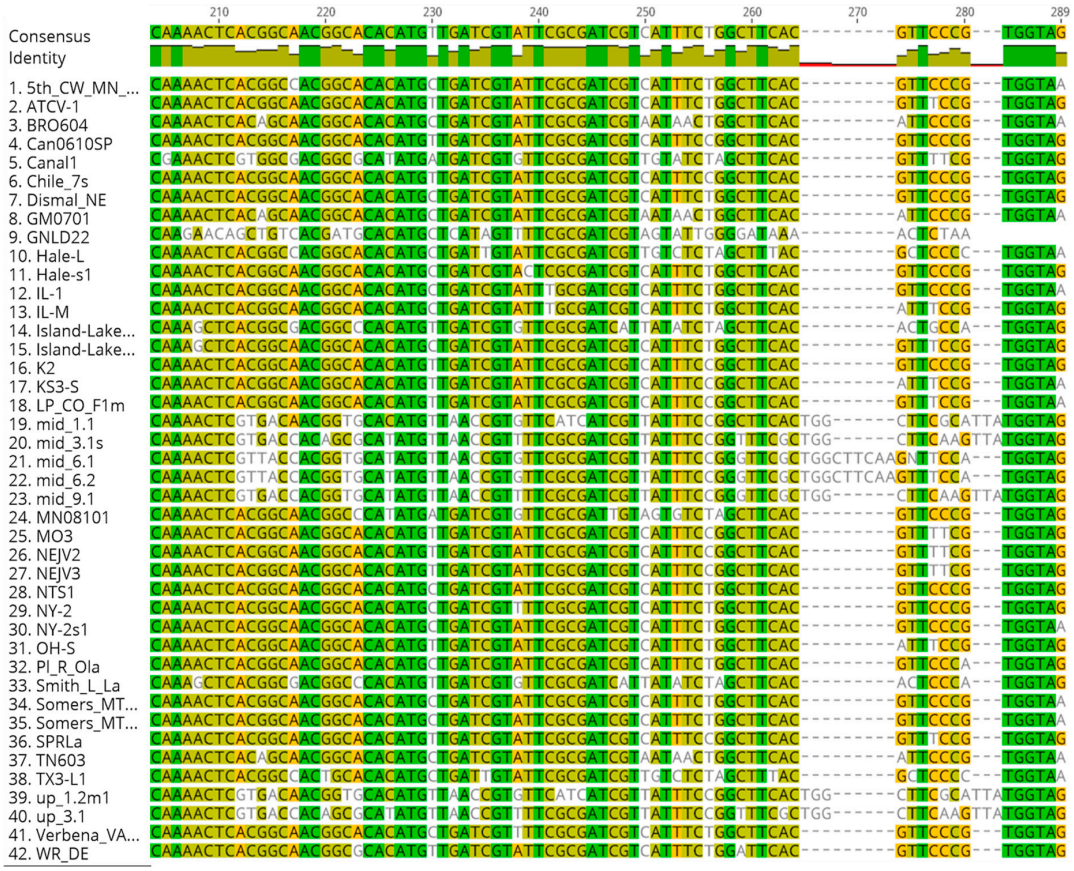

Figure A1. DNA sequence alignment of unique SAG chlorovirus *kcv* genes. DNA sequences were aligned using the Geneious Alignment algorithm from Geneious 11.0.5 software. Green color indicates identical nucletides among all sequenced strains. Different shades from olive to orange denote different degrees of conservation with olive color being the most conserved.

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