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Research paper

Evolution and postglacial colonization of Seewis hantavirus with *Sorex araneus* in Finland

Jiaxin Ling[a,*], Teemu Smura[a], Daniel Tamarit[b], Otso Huitu[c], Liina Voutilainen[a,d], Heikki Henttonen[d], Antti Vaheri[a], Olli Vapalahti[a,e,f], Tarja Sironen[a,e]

[a] University of Helsinki, Medicum, Department of Virology, Helsinki, Finland
[b] Uppsala University, Biomedical Centre, Science for Life Laboratory, Cell and Molecular Biology, Department of Molecular Evolution, Sweden
[c] Forest and Animal Ecology, Natural Resources Institute Finland, Tampere, Finland
[d] Forest and Animal Ecology, Natural Resources Institute Finland, Helsinki, Finland
[e] University of Helsinki, Department of Veterinary Biosciences, Helsinki, Finland
[f] Helsinki University Hospital, University of Helsinki, Helsinki, Finland

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ABSTRACT

Hantaviruses have co-existed with their hosts for millions of years. Seewis virus (SWSV), a soricomorph-borne hantavirus, is widespread in Eurasia, ranging from Central Siberia to Western Europe. To gain insight into the phylogeography and evolutionary history of SWSV in Finland, lung tissue samples of 225 common shrews (*Sorex araneus*) trapped from different parts of Finland were screened for the presence of SWSV RNA. Forty-two of the samples were positive. Partial small (S), medium (M) and large (L) segments of the virus were sequenced, and analyzed together with all SWSV sequences available in Genbank. The phylogenetic analysis of the partial S-segment sequences suggested that all Finnish SWSV strains shared their most recent common ancestor with the Eastern European strains, while the L-segment suggested multiple introductions. The difference between the L- and S-segment phylogenies implied that reassortment events play a role in the evolution of SWSV. Of the Finnish strains, variants from Eastern Finland occupied the root position in the phylogeny, and had the highest genetic diversity, supporting the hypothesis that SWSV reached Finland first form the east. During the spread in Finland, the virus has formed three separate lineages, identified here by correlation analysis of genetic versus geographic distance combined with median-joining network analysis. These results support the hypothesis that Finnish SWSV recolonized Finland with its host, the common shrew, from east after the last ice age 12,000–8000 years ago, and then subsequently spread along emerging land bridges towards west or north with the migration and population expansion of its host.

1. Introduction

Hantaviruses (genus *Orthohantavirus*, family *Hantaviridae*, order *Bunyavirales*, according to the new taxonomy (Briese and The ICTV Bunyaviridae Study Group, 2016)) are negative-sense single-stranded RNA viruses with a trisegmented genome. The genome consists of small (S), medium (M), and large (L) segments, encoding a nucleocapsid (N) protein, glycoproteins (Gn and Gc), and an RNA-dependent RNA polymerase, respectively. Hantaviruses are carried by host species belonging to Soricidae (shrews) and Talpidae (moles) families that both belong to Soricomorpha, Rodentia; More recently, hantaviruses have also been detected in Chiroptera (bats), all of which are globally distributed (Bennett et al., 2014; Zhang, 2014). Seewis virus (SWSV) is a widely spread soricomorph-borne hantavirus in Eurasia. It was first found in the lung tissue of Eurasian common shrews in Switzerland in 2006 (Song et al., 2007). Subsequently, genetically highly diverse SWSV strains have been detected in Austria, Croatia, Czech Republic, Finland, Germany, Hungary, Poland, Russia, Slovakia, and Slovenia (Gu et al., 2014; Gu et al., 2013; Kang et al., 2009a; Ling et al., 2014; Resman et al., 2013; Schlegel et al., 2012).

A special feature of *Sorex araneus* is its ubiquitous and rapid chromosomal evolution. The different chromosomal races (CR) of *S. araneus* are defined using karyotype variation and polymorphism. More than 60 largely geographically nonoverlapping CR have been described and classified into five distinct phylogroups: the Valais race, West European, East European, Siberian, and North European phylogenetic groups (Searle et al., 2010; Wójcik et al., 2003; Zhdanova et al., 2005). Evolutionary relationships in the *S. araneus* group, based on biochemical,

* Corresponding author at: Department of Virology, Faculty of Medicine, P.O. Box 21, Haartmaninkatu 3, FI-00014 University of Helsinki, Finland.
E-mail address: Jiaxin.ling@helsinki.fi (J. Ling).

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karyotypic, and sequence data analyses, have often been contradictory. These incongruences have been postulated as introgressions of mitochondrial DNA (mtDNA) or hybrid races (Bannikova and Lebedev, 2010; Yannic et al., 2008; Yannic et al., 2010). As a result, mtDNA or other genetic variations cannot serve as the sole reliable indicator of phylogenetic relationships between shrews. Instead, karyotypic changes can represent the evolutionary relationships among the species. (Lundqvist et al., 2011; Mackiewicz et al., 2017).

Due to recolonization of Fennoscandia (Finland and Scandinavia) from southwest and east after the retreat of the Weichselian glaciation, and subsequent glacial lake and sea phases (ca 18–7 thousand years ago (Hughes et al., 2016), common shrews in Fennoscandia belong to either the North European or West European groups (Fredga, 1996; Halkka et al., 1987; Halkka et al., 1994). The *eastern translocation cascade* is a commonly accepted hypothesis to explain the chromosomal evolution during the recolonization history of *S. araneus* in North Europe (Halkka et al., 1994). According to this hypothesis, Finnish groups are descendants of an old East European racial group that probably originated from east of the Urals. Most of Finnish groups formed during recolonization through consecutive events of Robertsonian fusion mutations (Searle, 1984). Six of the seven Finnish CRs (Sa: northern Finland, Ku: central Finland, Il: easternmost Finland, Lm: southeastern Finland, Ka: southern Finland Le: western Finland) evolved from a common source population east of Finland. These CRs are geographically adjacent or recently separated populations (Hausser et al., 1994). The seventh population, Abisko (Ai), is found in northwesternmost Finland and in northernmost Sweden (Fredga, 1996; Halkka et al., 1987; Halkka et al., 1994). The geographical range list of 7 Finnish CRs is demonstrated in Fig. 1.B. In addition to karyotypic variation, mitochondrial genetic variation suggests that shrews from southern parts of Finland differ from other Fennoscandian shrews, including shrews from Kuusamo and Muonio (defined in our study as central and northern Finland, respectively). Although nuclear and mitochondrial genetic variation is not congruent with chromosomal variation (Basset et al., 2006; Basset et al., 2008; Lundqvist et al., 2011), they both suggest that Finnish common shrews, possibly except the Ai-population, dispersed from the east (Lundqvist et al., 2011; Shchipanov and Pavlova, 2017).

Hantavirus evolution is thought to represent a process of virus-host co-divergence over a time-scale of millions of years, combined with cross-species host switches at multiple scales (Holmes and Zhang, 2015; Plyusnin and Sironen, 2014). Consequently, based on the evolutionary history of *S. araneus* and the postglacial geological history of Finland, we hypothesize that all Finnish SWSV, except for the northwesternmost lineage, originated from the east and then subsequently spread along emerging land bridges towards west or north. In our previous study, we presented two evolutionary scenarios regarding the phylogeny of the S- and L-segments of the SWSV genome (Ling et al., 2014). Based on the S-phylogeny, Finnish SWSV could be a unique subtype compared to the other European SWSV strains. However, based on the L-phylogeny, two sublineages circulate in southern and northern Finland (Lapland). To further elucidate the geographical origins, diversification and phylogeographic patterns of SWSV, we screened 225 common shrews from 36 localities around Finland (Fig. 1A). We obtained a better insight of the variability of the SWSV, as well as the recolonization routes of SWSV into Finland using phylogeographic and network analyses.

2. Materials and methods

2.1. Sample collection

Altogether 225 common shrews were collected from 36 localities covering all of Finland (except Åland Islands and northernmost Lapland) in 2013 and 2014 (Fig. 1A, Table S1). Small mammals were either snap- or live-trapped using either standard snap traps or Ugglan livetraps. The animals were either dissected immediately and samples frozen or animals were frozen on dry ice in the field and dissected later. During dissection, the standard information (species, age, sex, weight, breeding status) was collected. Lung samples were stored at − 70 °C until further processing.

2.2. Ethics statement

Permit (7/5713/2013) for capturing protected species (all shrews are protected in Finland) was granted by the Finnish Ministry of the Environment. No ethical permit is needed for snap- and live-trapping in Finland.

2.3. Total RNA extraction, RT-PCR and sequencing

The total RNA extraction and RT-PCR procedures were performed as previously described (Ling et al., 2014). The RNA was stored at − 20 °C for up to one week or at − 70 °C for longer storage. All lung tissue samples were tested first for the presence of hantaviral RNA using semi-nested RT-PCR that amplifies partial L-segment sequence (Klempa et al., 2006). Oligonucleotide primers were designed by using all available SWSV sequences for amplification and sequencing of the genome (Table S2).

PCR was performed in 20-μl reaction volume using the Phusion Flash High-Fidelity PCR Master Mix (Thermo Scientific). PCR-products were separated by electrophoresis on 1.5% agarose gels and purified using the QIAQuick Gel Extraction Kit (Qiagen, Hilden, Germany). Some of the viral amplicons were directly sequenced and some were cloned into a vector (pJET1.2/blunt) by CloneJET PCR Cloning Kit (Thermo Scientific) and sequenced with pJET1.2 Forward and Reverse Sequencing Primers. The exact nucleotide sequences of the 5′ and 3′ termini of the S segment sequences were determined using the RNA ligation method in (Li et al., 2013). All virus genome sequences generated in this study have been deposited in GenBank under the accession numbers KY651020-KY651083.

2.4. Dataset

New sequences were assembled and included into the datasets of 97 S-segment ORF sequences, and 89 partial L-segment sequences, downloaded from the National Center for Biotechnology Information (NCBI) website (http://www.ncbi.nlm.nih.gov/). Sequences were aligned with Mafft-linsi (v. 7.305) (Katoh et al., 2002), and the alignments were trimmed by removing columns with over 50% gaps using trimAl (v. 1.2.rev59) (Capella-Gutierrez et al., 2009) as well as sequences shorter than 50% of the alignment length using custom perl scripts. The final datasets referred here as ‘the global SWSV’ and ‘the local SWSV’. The ‘global SWSV’ dataset included 111 partial ORFs of S-segment; 13 partial M-segments, and 129 partial L-segments (Table 1). The local dataset included Russia, Polish and Finnish SWSV sequences (For Finnish datasets, it includes 28 partial ORFs of S-segment, 13 partial M-segment, and 62 partial L-segments, (Table S1)). For all datasets, we first detected the recombination events by using the Phi-test in SPLITS TREE 4.0 (Huson and Bryant, 2006), and the alignments without recombination events ($p < 0.01$) were proceeded to the phylogenetic analysis.

2.5. Phylogenetic analysis

Phylogenetic trees were inferred using the Bayesian method implemented in MrBayes v3.1.2 (Ronquist and Huelsenbeck, 2003), using the General Time Reversible (GTR) model, determined by jModelTest (Darriba et al., 2012), and a 4-category gamma-distribution model of among-site rate heterogeneity for all the alignments. MrBayes was run for 10 million generations for the S- and M-segment sequences, and 12 million for the L-segment sequences, with final standard deviations between 2 runs of 0.0058, 0.0009 and 0.0071, respectively. The reconstruction parameters with the S sequences yielded a median

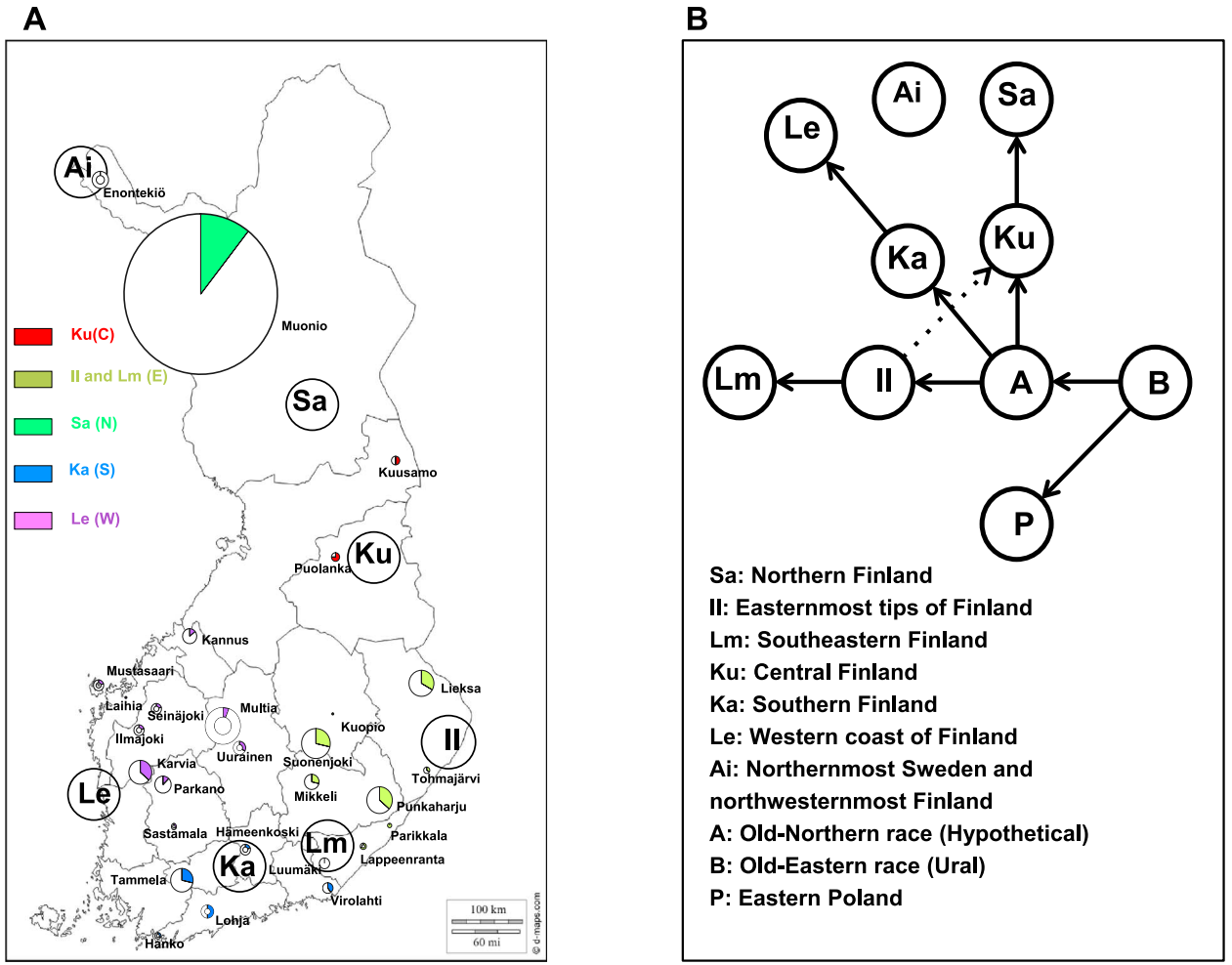

Fig. 1. A RNA prevalence and distribution of Finnish SWSV samples: Doughnut charts, -SWSV from a previous study (Ling et al., 2014); Pie charts - new isolates. Chart sizes represent the number of animals tested; the smallest charts (Laihia, Kuopio) = 1, the largest (Muonio) = 75 individuals.

The distribution of chromosomal races of *S. araneus* in Finland (Zima et al., 1996): Sa (N): northern Finland (in green) (Distribution: Lapland, N Finland), Ku(C): central Finland (in red) (Distribution: central Finland around the northern border of the central Finnish Lake District), Il: easternmost Finland (in yellow) (Easternmost tip of Finland), Lm: southeastern Finland (in yellow) (Distribution: The southern bank of Lake Saimaa, eastern parts of the Salpansselka Ridge, SE Finland), Il and Lm (E), Ka (S): southern Finland (in blue), Le (W): western Finland (in purple) (Distribution: Åland, the mainland of western Finland) and the Abisko karyotype, Ai, at Kilpisjärvi. The seventh karyotype, Abisko (Ai), is found in northwesternmost Finland and in northernmost Sweden, and is from a different origin (Halkka et al., 1994).

B Colonization routes and the hypothesis on chromosomal evolution of *S. araneus*, modified from (Halkka et al., 1994; Wojcik et al., 2003; Zima et al., 1996). Eastern translocation cascade to north European chromosomal races theory: Finnish karyotype Ku and Sa may have evolved either directly or via FIN karyotype Il from an old northern karyotype (arm pool), the old northern karyotype has given rise to FIN karyotype Ka and FIN karyotype Il; FIN karyotype Lm derived from karyotype Il. However, based on the mitochondrial DNA, S Finland karyotype/haplotypes are later colonizers, appearing probably during the Litorian Sea phase, 8000–7000 BPR (Björck, 1995; Lundqvist et al., 2011). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Seewis strains analyzed in the study.

Segment	Country	N	Sampling dates	Reference
S	Czech Republic	45	2004–2010	(Schlegel et al., 2012)
	Germany	3	2008	(Schlegel et al., 2012)
	Hungary	7	1997–2000	(Kang et al., 2009a)
	Poland	3	2010–2013	(Gu et al., 2013, Gu et al., 2014)
	Slovakia	1	2008	(Schlegel et al., 2012)
	Switzerland	1	2006	(Song et al., 2007)
	Slovenia	6	2013	(Resman et al., 2013)
	Russia	15	2007–2008	(Yashina et al., 2010)
	Finland	30	1982–2014	(Kang et al., 2009a, Ling et al., 2014) and this study
L	Czech Republic	28	2004–2010	(Schlegel et al., 2012)
	Germany	4	2008	(Schlegel et al., 2012)
	Hungary	1	1997–2000	(Kang et al., 2009a)
	Poland	7	2010–2013	(Gu et al., 2014, Gu et al., 2013)
	Slovakia	1	2008	(Schlegel et al., 2012)
	Switzerland	2	2006	(Song et al., 2007)
	Slovenia	6	2013	(Resman et al., 2013)
	Russia	13	2007–2008	(Yashina et al., 2010)
	Finland	64	1982–2014	(Ling et al., 2014, Kang et al., 2009a) and this study
	Croatia	1	2013	

effective sampling size (ESS), “avgESS” of 6005 (lowest minimum ESS, “minESS” of 3401); the M sequences yielded a median avgESS of 10327 (lowest minESS of 6566); and the L sequences, a median avgESS of 2860 (lowest minESS of 1269). All parameters had associated Potential Scale Reduction Factor (PSRF) values higher than 0.9997.

Trees were also reconstructed using IQ-TREE v. 1.5.5 (Trifinopoulos et al., 2016) with automatic model selection (Kalyaanamoorthy et al., 2017) and branch supports were assessed by 1000 ultrafast bootstrap (Minh et al., 2013) pseudoreplicates and SH-like approximate likelihood tests (Guindon et al., 2010).

Ancestral geographical distributions of each lineage were reconstructed using Bayesian Markov chain Monte Carlo (MCMC) method implemented in BEAST package version 2.4.2 (Drummond and Rambaut, 2007) as the same method described in (Souza et al., 2014). For the Finnish dataset, the trapping locations were labelled according to the distribution of the chromosomal races, since a given karyotype is found in a restricted geographical region; the labels were karyotype Ka (Southern Finland), karyotype Le (Western Finland), karyotype Il and Lm (Eastern Finland), karyotype Ku (Central Finland), and karyotype Sa (Northern Finland) (Zima et al., 1996; Wójcik et al., 2003; Halkka et al., 1987; Halkka et al., 1994). The same evolutionary model was employed as described above with strict clock model, and the Yule model as the tree prior. The analyses were performed with two independent chain that were combined using the LogCombiner v1.4.7. Maximum clade Credibility (MCC) trees were constructed using the TreeAnnotator program, and visualized in the FigTree (version 1.4.2).

2.6. Median-joining network analysis

The MJ networks were constructed from three alignments including 28 ORF of the S-segment (1290 bp), 13 partial M-segment, and 62 partial L-segment sequences, using SPLITS TREE 4.0 with Epsilon 1 and 2000 spring embedded iterations. A NeighborNet split-graph was constructed using an alignment of the S-segment from 28 SWSV strains, as well as a concatenated alignment of S- and L-segment sequences from 28 SWSV strains (1608 bp). The potential recombination events were sought for all the alignments using the Phi-test in SPLITS TREE 4.0 (Huson and Bryant, 2006).

2.7. Correlation between geographic and genetic distances matrices

Pairwise genetic distances between SWSV strains were calculated in MEGA 7. Analyses were conducted using the Tamura-Nei nucleotide substitution model, calculated in MEGA. The rate variation among sites was modeled with a gamma distribution (shape parameter = 1). The geographic distances between trapping locations were calculated using Google Earth (https://www.google.com/earth/). All matrices were analyzed with R Studio (R) (R Development Core Team, 2008). The package ‘Vegan’ was used for the Mantel tests (Oksanen et al., 2017), which are used to test for correlation between two or more distance matrices (Lichstein, 2007). Mantel tests were conducted for the S and L-segment, comparing genetic and geographic distances. The *Mantel r* above the zero exhibit positive spatial autocorrelation, those below have negative autocorrelation. Linear regression analysis was conducted in GraphPad Prism (version 6.0).

3. Results

3.1. Detection of SWSV in Finnish shrews

Based on the RT-PCR detection of SWSV followed by sequencing of the partial L-segment, 42 (18.7%) common shrews from 14 locations, out of 225 common shrews from 36 locations, were positive for SWSV. This included 8 positive out of 75 common shrews (10.7%) from Muonio (N Finland) and 34 positive out of 150 common shrews (22.7%) from the other locations in Finland. For each location, the SWSV RNA prevalence can be found in Fig. 1.

Forty samples were subjected to RT-PCR specific for SWSV S-, M-, and L-segments (two samples were excluded because of the low sequence quality in the initial screening). The sequences of complete coding region of 14 S-segments from nine locations in five provinces of Finland were obtained, including two from Eastern Finland (Punkaharju, Parikkala), three from Western Finland (Karvia, Kannus, Parkano), five from northcentral Finland (or Oulun lääni) (Puolanka, Kuusamo) and three from northern Finland (Muonio). In addition, seven partial M-segments (1200 nt) and four partial L-segments (1190 nt) were sequenced (Table S1, supplementary files).

3.2. Genetic characterization of Finnish SWSV

As no full genome of SWSV was available, we attempted to sequence one, and were able to obtain full length S- and M-segment sequences from the strain EWS25 (Tammela, Southern Finland, 2012). The S-segment comprised of 1641 nt. As expected, the 3′-terminal nucleotide sequence (3′-AUCAUCAUACGAGGG) was complementary to the 5′-terminal sequence (5′-UAGUAGUAGACUCCC), and consistent with the panhandle secondary structure of hantaviral RNA. The S-segment ORF was 1290 nt long (corresponding to positions 47–1336 of the complete S-segment sequence of SWSV, Genbank Acc. No. EF636024), encoding a putative N protein of 429 amino acids (aa).

The M-segment sequence consisted of 3533 nt, and it had a single ORF (41–3460 nt) encoding a putative GPC protein of 1139 aa. A putative signal peptide of 23 aa in the beginning of the ORF, and the ^{648}WAASA^{652} motif determining the cleavage of GPC into the Gn (630 aa) and Gc (487 aa) glycoproteins were identified (Hepojoki et al., 2012). Zinc finger domains (549–595 aa) and the ^{619}YxxL^{622} motif were also identified on the glycoprotein of the EWS25 strain.

Only a partial L-segment sequence (1200 nt), corresponding to positions 1144–2343 of the complete L-segment sequence of Asikkala virus, host *Sorex minutus*, (Genbank Acc. No. KC880349), could be determined due to limited tissue material available for the analysis.

3.3. Phylogenetic analysis of the SWSV

To investigate the phylogenetic relationship of all SWSV strains,

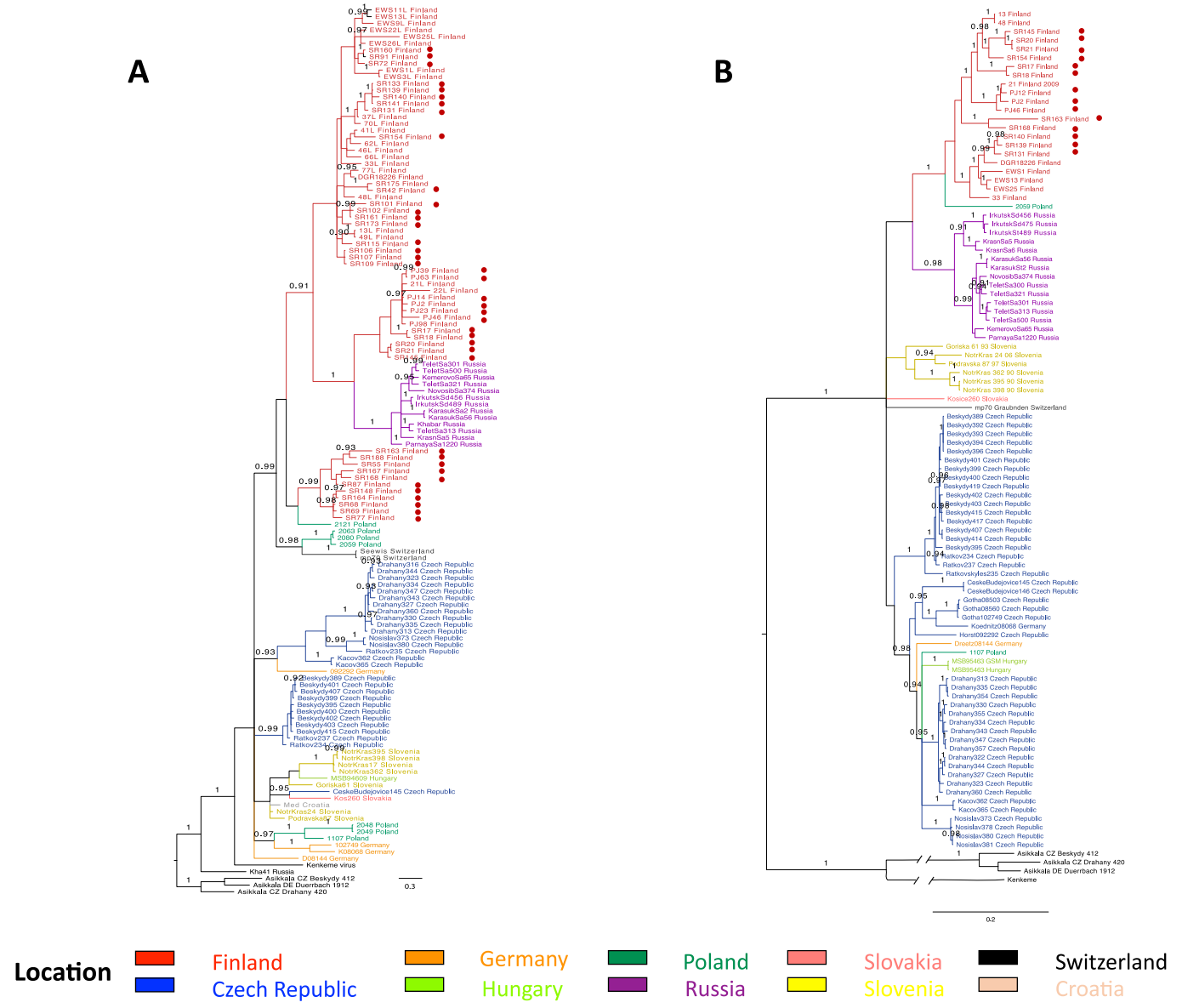

Fig. 2. Bayesian trees based on the partial L- (A), and S-segment (B) of all extant SWSV. The trees were generated by MrBayes 3, using the best-fit GTR + G model of evolution as estimated from the data by jModeltest, based on the alignment of the 317-nucleotide L-, and the 1197-nucleotide S-segment sequences; posterior probability values of over 0.9 are shown on the branches. The scale bar shows the number of nucleotide substitutions per site.

Bayesian trees and Maximum-likelihood trees were constructed on the basis of the partial L segment sequences (317 nt, N = 133), and partial ORF of the S segment sequences (1197 nt, N = 100) rooted on the Asikkala and Kenkeme viruses (Fig. 2A, B and Fig. S2.A, B). The topology of the Bayesian trees (Fig. 2) and Maximum-likelihood trees (Supplementary Fig. S2) were similar. On the basis of the partial L-segment tree, the Finnish strains grouped in three different lineages. The strains from central or northern Finland grouped together with strains from Russia (Western and Eastern Siberia). The strains from western Finland and some strains from southern and eastern Finland formed a distinct cluster, while the rest of the strains from southern and eastern Finland clustered together with a Polish strain (SWSV 2121, Kurowice, Genbank Acc. No. KC537794). The Polish strains (in turquoise in Fig. 2) had the highest diversity: SWSV 1107 (capture site: Boginia, JX990967), SWSV 2048 (Huta Dłutowska, JX990944), and SWSV 2049 (Huta Dłutowska, JX990945) shared the most recent common ancestor with German strains, while the other Polish SWSV strains (SWSV 2059, capture site: Chimiel, JX990941; SWSV2063, Chimiel, JX990942; SWSV 2080, Chimiel, JX990943) clustered together with strains from Switzerland. SWSV from Czech Republic formed a monophyletic cluster and strains from Slovakia, Hungary, Slovenia, and Croatia were mixed together in a distinct cluster.

On the basis of the S-segment, all SWSV fell into three lineages (Fig. 2.B). All Finnish strains shared a most recent common ancestor with one Polish strain (2059). In contrast to L-segment phylogeny, the Russian strains formed a distinct cluster and the strains from central Finland and Lapland clustered together with the other Finnish SWSV strains. The strains from Central Europe (Poland (1107), Hungary, Czech Republic, Germany, Slovenia, Slovakia and Switzerland) clustered together.

3.4. Phylogenetic analysis of Finnish SWSV

On the basis of the L-segment, the Finnish SWSV strains originated from one source population and they shared a deep node with the strain 2121 from Poland (Fig. 3.A and Fig. S3.A). Furthermore, the diversification of Seewis appeared paraphyletic. The cluster L1 consisted exclusively of strains from eastern Finland (more specifically, the Karelian

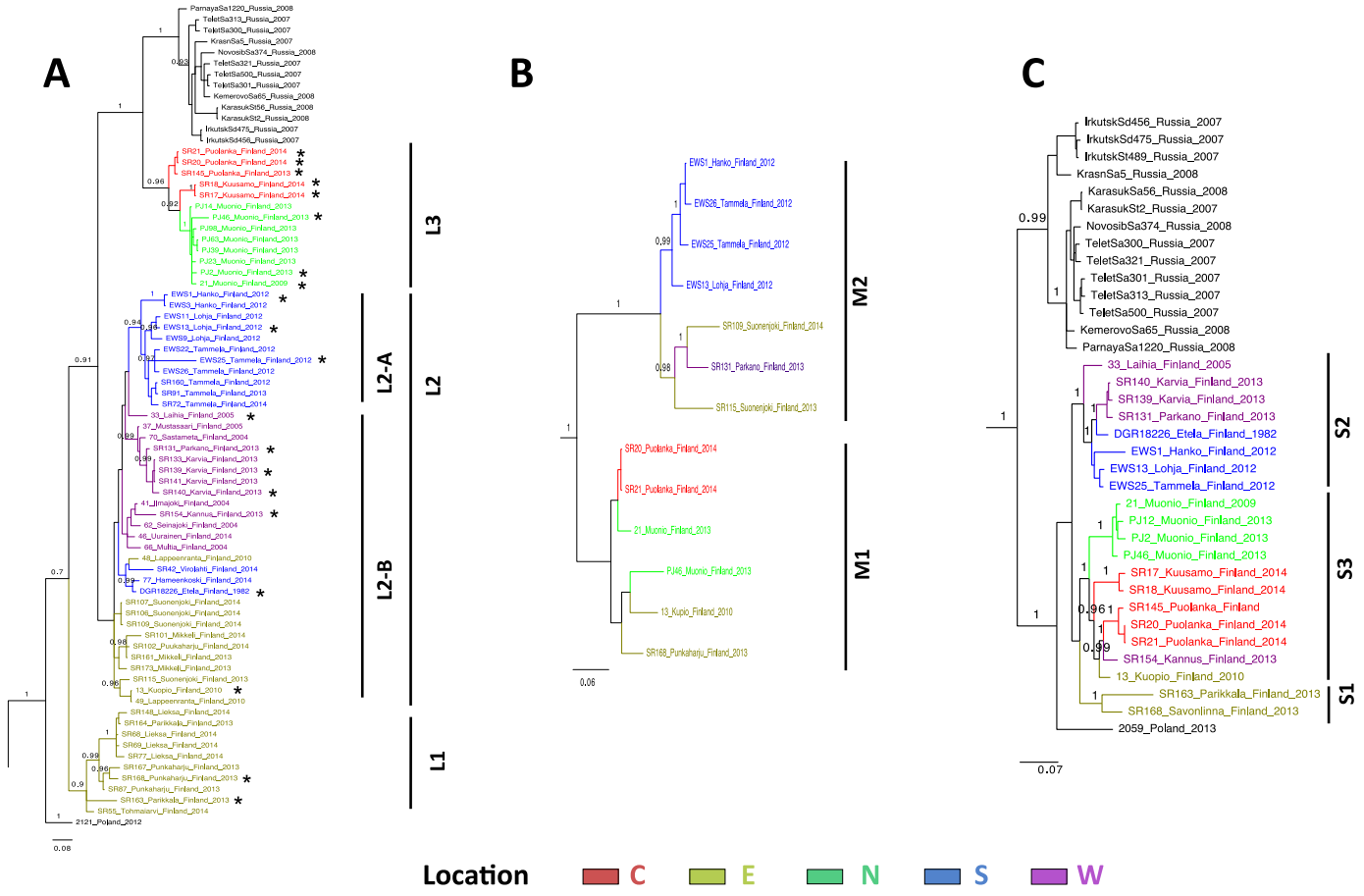

Fig. 3. Bayesian trees based on the partial L-(A), M- (B), S- (C), segment sequences of Finnish SWSV. Bayesian trees were generated by MrBayes 3, using the best-fit GTR + G model of evolution as estimated from the data by jModeltest, Five districts: southern Finland (S), western Finland (W), eastern Finland (E), Oulu Finland (C), and Lapland Finland (N) were assigned to each sequence. Posterior probabilities values of over 0.9 are shown on the branches. The scale bar shows the number of nucleotide substitutions per site.

region). L2 could be further divided into two sub-clusters, of which, L2-A contained strains from southern Finland (Hanko, Lohja and Tammela), whereas L2-B contained strains from southern, western and eastern Finland. Within L2-B, there were smaller monophyletic clusters with geographic structure (i.e. three clusters in western Finland; south-eastern/southern cluster and a Savonia-region cluster (eastern Finland)). The cluster L3 contained strains from northern and central Finland and clustered together with SWSV strains from Western Siberia.

On the basis of the S-segment, all Finnish strains formed one monophyletic cluster that shared a common ancestor with the strain 2059 from Poland (Fig. 3.C and Fig. S3.C). Further, all these strains shared a common ancestor with the strains from western Siberia. The Finnish strains could be subdivided into three clusters, designated here as S1, S2, and S3. S1 contained strains from the eastern Finland. S2 contained strains from southern and western Finland, and S3 strains from northern and central Finland as well as one strain from western Finland (Kannus) and one strain from eastern Finland (Kuopio).

Notable differences were seen in the topology of the L- and S-segment trees. For example, strains from L3 (northern/central Finland) shared ancestry with Russian strains in the L phylogeny (Fig. 3.A, and Fig. S3.A), while in the S phylogeny, they shared ancestry with the strains from L2 (southern/western Finland and Savonia), and together, formed the cluster S2 (Fig. 3.C, and Fig. S3.C). Furthermore, while the southern Hanko/Lohja/Tammela strains were monophyletic both in S (S2) and L (L2-A) phylogenies, some strains that grouped together with southern strains in L2 cluster (such as 13 and SR145) were placed in S3 cluster (i.e. together with strains from central and northern Finland). These differences suggest multiple reassortment events during the spread of SWSV in Finland.

In order to assure that the differences between the L- and S-segment sequence based phylogenies were not only due to having different taxon sets for each of them, we reanalyzed both segments using strains of which both L- and S-segment sequences are available (Fig. S4A, and B). The clustering pattern remained similar; i.e. on the basis of S-segments all Finnish strains cluster together with the Polish strains 2059 and 2063, whereas on the basis of L-segments the strains from northern and central Finland clustered together with western Siberian strains.

Since no other SWSV M-segment sequence in the GenBank could be aligned with our dataset, all Finnish strains grouped together, and were further divided into two clusters, of which M1 contained the two strains from eastern Finland and the strains from northern and central Finland, whereas M2 contained strains from eastern, western, and southern Finland (Fig. 3.B, and Fig. 3S. B). Again, the tree topology of M-segments differed from those of the S- and L-segment trees. For example, the topology of the M2 cluster resembled that of the L2, whereas M1 contained strains that grouped into clades S1 and S2 on the basis of S-segment, and to L1, L2-B and L3 on the basis of L-segment.

In order to understand the post-glacial recolonization, and spread of SWSV in Finland, we inferred the posterior root state probabilities at the nodes of Bayesian trees for S- and M-segment but not L-segment, since the L-phylogeny is paraphyletic. In S- and M-phylogeny, the eastern strains displayed the best-defined root location, with a probability of 34.0% and 28.0%, respectively (Fig. S3.B, C).

3.5. Network analysis

To further test the reassortment, recombination and evolutionary history of Finnish SWSV strains, we constructed unrooted phylogenetic

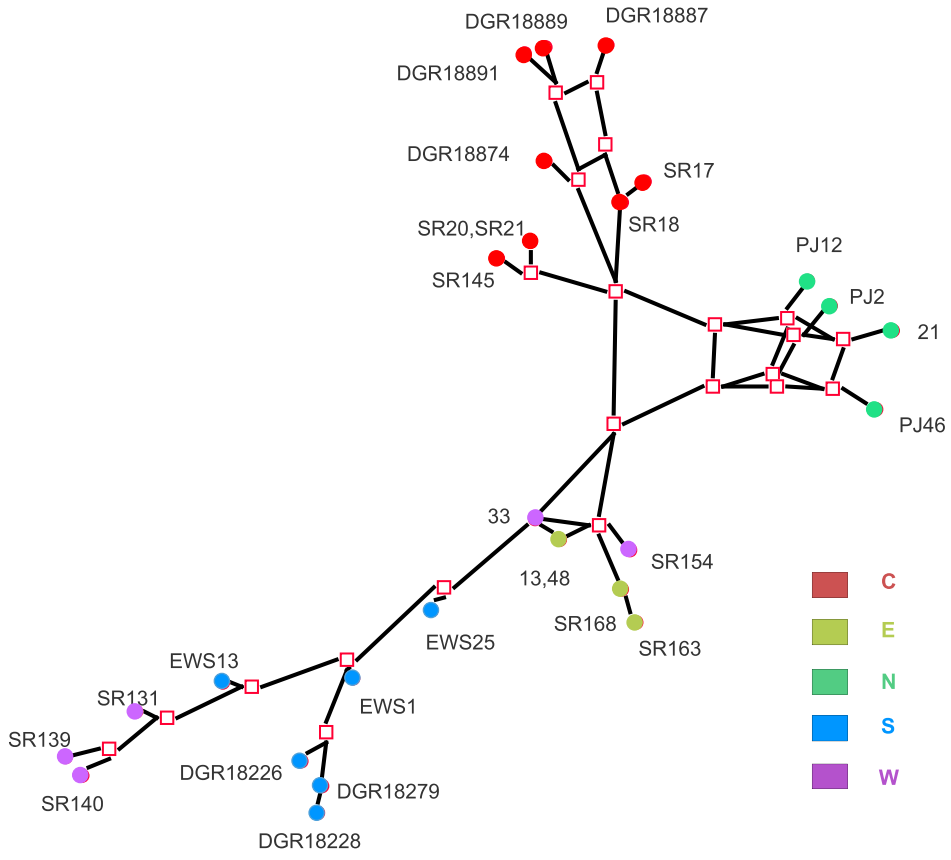

Fig. 4. MJ network. Plot of a 1290 character set of 28 SWSV S-segment. Different colours representing the isolates' location. MJ network is showing three separated lineages. The strains from central and north are connected, and strains from south and west are related with each other.

networks from three alignments (Fig. 4, and Fig. S5.A, B). Consistent with the Bayesian trees and MCC trees (Fig. 3 and Fig. 3S), three separate lineages were detected (Fig. 4, and Fig. S5.A, B). Within the lineages, the strains from central and northern Finland were connected, and strains from southern and western Finland were related in the partial S, M, and L alignments. No recombination events were detected in the S-, M- or L-segment alignments according to the Phi-test. However, in a concatenated alignment of 28 partial S- and L-segment sequences, the Phi-test did find statistically significant evidence for reassortation (p = 0.02) (Fig. S5.A and B) supporting the reassortment events suggested by the Bayesian trees (Fig. 3 and Fig. S3).

3.6. Correlation between geographic and genetic distances

To investigate the significance of the correlation between genetic and geographic distance of SWSV in Finland, we analyzed the correlation between these two matrices for both the L- and S-segment by using the Mantel test. The *Mantel r* for L- and S-segment were 0.5024 (Fig. 5.A) and 0.5898 (Fig. 5.B) respectively (p value is 0.001). Within a geographic region (analysis based on the L segment), positive correlations were observed for the SWSV strains from eastern Finland (*Mantel* $r = 0.4598$), and western Finland (*Mantel* $r = 0.473$), as well as southern Finland (*Mantel* $r = 0.2796$). For the S-segment, positive correlations were observed for the SWSV strains from eastern Finland (*Mantel* $r = 0.5433$), western Finland (*Mantel* $r = 0.3741$), and southern Finland (*Mantel* $r = 0.4734$) (p = 0.001) (Fig. 5.C). The genetic distance of partial L-segment ranged from 0 to 0.317 in eastern Finland, while in the other geographic regions, lower local genetic diversities were observed: 0.006–0.213 in south Finland, 0–0.013 in central Finland, 0–0.126 in northern Finland, and 0–0.02 in western Finland. Among the strains from Eastern Finland, there was a lack of correlation for the L-segment sequences between the genetic and geographic distances. In contrast, when analyzing the L-segment sequences of the strains from the other regions (western, central and southern Finland), the genetic distance increased with geographic distance, but in a non-linear manner.

To test the directionality of spread, we used the strain SR168 (from Punkaharju, South-Eastern Finland) as a standpoint, according to the topology of the SWSV phylogeny that placed SR168 in the basal lineage (Fig. 3.D). All other Finnish SWSV strains (S-segment sequences) were then plotted against this standpoint. For the S-segment, the genetic distance increased with geographic distance linearly, but apparently, with two slopes (east to southern/western Finland and east to central/northern Finland) (Fig. 5.D). When these two groupings were plotted separately (Fig. 5.D), and the correlation was assessed with linear regression, relatively high R^2 values, 0.7125 and 0.8852, respectively, were observed (Fig. 5.E and F). Intriguingly, an outlier (SR154 from Kannus, located in the North Central Ostrobothnia; Fig. 1.A) was noted among the strains from western Finland. Consistently with the phylogenetic tree, this strain seems to fit better in the eastern than to central/northern Finland slope (Fig. 5.F). Therefore, genetic distance per kilometer is lower for the cluster S2 than for S3 (Fig. 3.C and Fig. S3.C), suggesting that the viability of Seewis S-gene in the southern/western Finland is higher than those in the central/northern Finland.

4. Discussion

We present here a detailed insight to the evolutionary history of SWSV with its host, the common shrew. To this end, we captured shrews from all of Finland, and detected the virus in 18.7% of the shrews. The genetic analysis of these SWSV strains suggested geographic structure on the basis of all three segments, but clear differences between the phylogenetic tree topologies of the different segments. Altogether the results suggest that the postglacial geographic spread of SWSV was combined with a complex reassortation pattern.

Finland was recolonized by flora and fauna mainly from the east

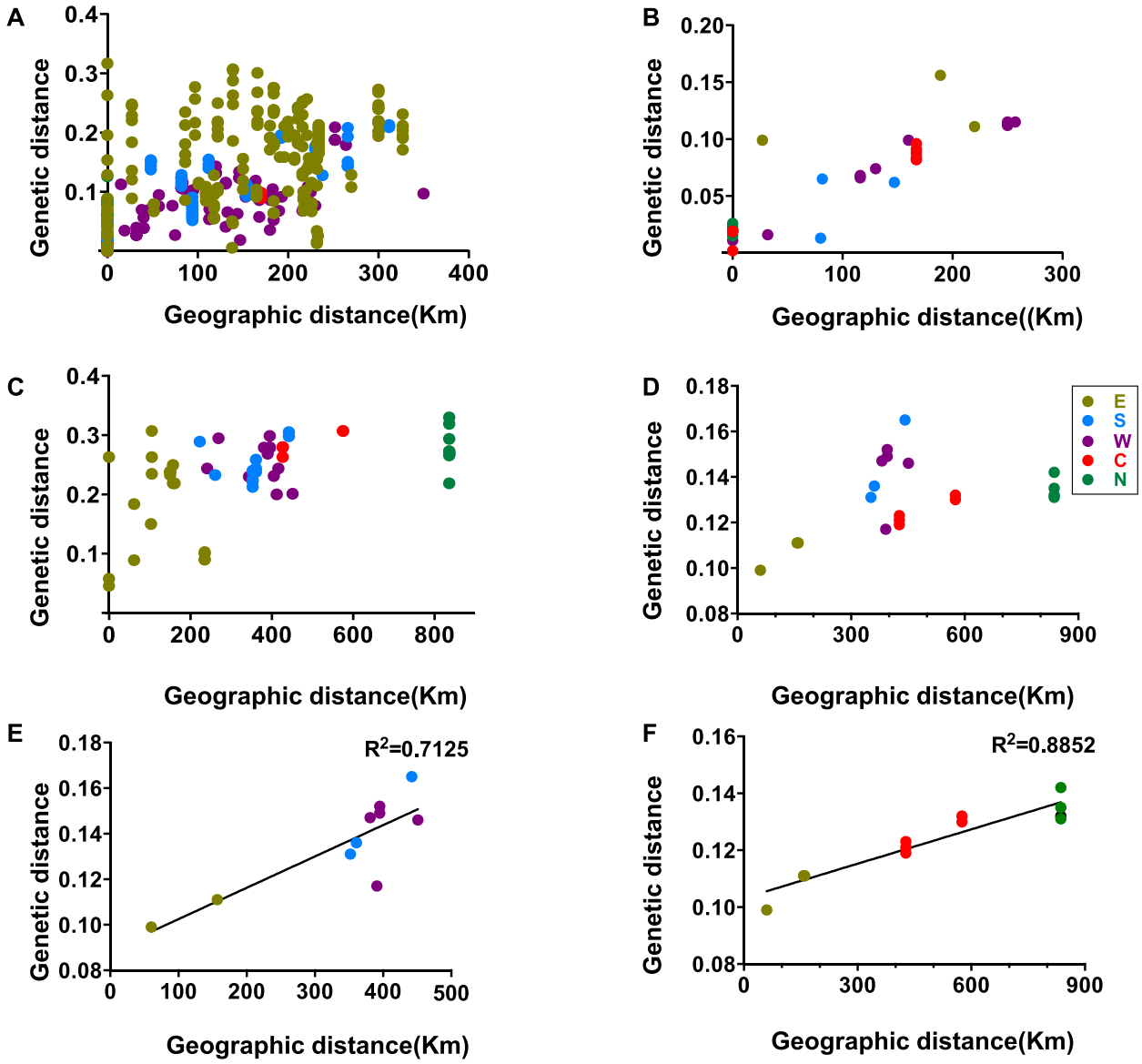

Fig. 5. Pairwise genetic versus geographic distance plot of Finnish SWSV L-segments (A) and S-segments (B). Genetic versus geographic distance plot between SR168 (Savonlinna/Punkaharju), and the other Finnish SWSV strains for the L-segment (C) and S-segment (D). Linear regression of SR168 plotted with southern, western, and eastern SWSV (E) and northern and central SWSV (F). Correlation was assessed with *Mantel r*. For the Finnish dataset, we used the data of SWSV strain SR168 as the standpoint to measure the SWSV dispersal since its location (Savonlinna/Punkaharju) is close to the eastern border of Finland and is placed in the basal lineage of the phylogenetic trees.

and southeast after the Last Glacial Maximum (LGM), when the ice retreated 17,000–10,000 years ago (Andersen and Borns, 1997; Hughes et al., 2016) and dry land subsequently emerged. Several species, for instance bank voles (*Myodes glareolus*), the host of Puumala hantavirus (PUUV), recolonized Fennoscandia from the east over Finland to northern Sweden and northern Norway, and from southwest to southern and central Sweden and Norway (Jaarola et al., 1999; Tegelström, 1987). These recolonization routes are still reflected in the phylogeny of PUUV (Asikainen et al., 2000; Razzauti et al., 2009). Common shrews are thought to have spread to Finland via the first land bridges, and the present distribution of chromosomal races of *S. araneus* can be mostly explained by the Ancylus Lake gulf system ca. 9000–8000 years ago (Björck, 1995; Halkka et al., 1987). *S. araneus* spread first to easternmost Finland, and further north and west along the land bridges. Finnish karyotypes evolved during this process according to the basic chromosome arm combinations of the metacentric chromosomes. We found here that SWSV strains from eastern Finland presented with a higher genetic diversity (up to 0.317% nt diversity) than the strains from the other regions in Finland (0.126%) (Fig. 5). This suggests that the virus may have circulated for a longer time period in eastern Finland, which is consistent with the recolonization route of *S. araneus*, and that eastern Finland thawed and was above the water level much earlier than southern and western Finland (Björck, 1995; Svendsen et al., 2004) (Fig. S1).

On the basis of karyotype and mitochondrial variation, it has been proposed that southern Finland was colonized by the common shrew later than northern Finland, and from a different part of the source population (Halkka et al., 1987; Lundqvist et al., 2011). The phylogeny of the S-segment sequences suggested that all Finnish SWSV S-segments formed a monophyletic group that shares a common ancestor with a Polish SWSV strain. A plausible scenario would suggest that these strains share a common ancestor (potentially from Ural) that has dispersed to Europe together with its host during last post-glacial period. This is supported by the equal genetic distance of all Finnish strains (including the northern strains) to Polish strains (Hypothesis A, Fig. S1). An alternative hypothesis would be that Finnish SWSV S-segment

lineages would be derived from a central European refugial location and spread northwards after the LGM (Hypothesis B, Fig. S1). More SWSV samples should be sequenced in nearby countries (e.g. Baltic region, Russian Karelia and Scandinavian countries) to retrace the history and infer the exact dispersal routes of SWSV into Finland (Fig. S1).

The phylogeny of the SWSV L-segment showed intriguing differences from that of the S-segment. On the basis of the L-segment, the Finnish SWSV strains formed three clusters, shared (consistently with S-segment phylogeny) a common ancestor with a Polish strain, whereas L3 shared, in contrast to S-segment phylogeny, a common ancestor with Russian strains from western Siberia (east of Urals) (Yashina et al., 2010). Although based on the Fig. 2A, it appears that the direction of SWSV diversification was from Finland to Russia, this is unlikely to be taken into consideration as the migration routes of the shrews in (Hypothesis C, Fig. S1). Rather it seems that L3 shared a more ancient ancestry with Russian strains, which is agreed in (Fig. S3.A). The eastern population seems to be the source of all Finnish SWSV, and with further sampling, L3 would be found there. The cluster L2 (with no known close relatives from other countries) contained strains from southeastern/eastern Finland, southern Finland, and western Finland. These results suggest that the SWSV L-segment may have dispersed to Finland via (at least) two routes; from southeast along the Karelian Isthmus, and earlier via a more eastern route, north of the present big lakes Ladoga and Onega, to central/northern Finland.

The difference between the L-segment and S-segment phylogenies imply reassortation events. Such events have been reported for a few hantavirus species, and recently also for Imjin virus, which is a shrew-borne hantavirus (Lee et al., 2017). The correlation analysis between genetic and geographic distances suggests that the S-segment may have dispersed from southeast towards north. Hypothetically, this may have been followed by a reassortation event between 'southeastern S-segment' and 'northern L-segment'. Such re-assortment events would be analogous to those observed previously with PUUV in Finland (Razzauti et al., 2009). The current dataset suggests that this reassortant may have outcompeted the original 'northern S-segment' lineage, since, so far, all SWSV strains sequenced from the northern Finland group together in a clade, where SWSV strains from south-eastern Finland form the basal cluster.

The M-segment sequence dataset available for the analysis was scarce. However, the general evolutionary pattern of M-segment seems to reflect those observed in S-segment and L-segment phylogenies. Also with the M-segment, the highest genetic diversity was observed in eastern Finland. A strain from south-eastern Finland (Punkaharju) was a basal lineage for the eastern/norther cluster and the strains from Suonenjoki, eastern Finland grouped together with the strains from southern and western Finland.

Altogether, the genetic and phylogenetic analyses suggest that the Finnish SWSV strains were introduced from east of Finland, from where they underwent further dispersal to the north and to south/west. This phylogeographic structure of SWSV reflects the above mentioned postglacial recolonization pattern of *S. araneus* (Halkka et al., 1987; Lundqvist et al., 2011). However, it should be noted that original source population of SWSV was apparently not homogenous, but, more likely, contained different L-segment lineages. The S-segments of the Finnish SWSV strains showed more clearly a geographic structure and exhibited linear correlation between genetic and geographical distances. Notably, assuming a southeastern SWSV strain (that is in the root position of S-segment phylogenetic tree) as a standpoint, the analysis suggested two different slopes for linear correlation. This was further confirmed by using a sequence from the west was used as the standpoint, resulting in much lower correlation (data not shown).

The hypothesis of co-evolution between hantaviruses and their hosts is based on their co-phylogeny, as inferred from genetic markers including chromosomal and mitochondrial phylogeny. While testing this hypothesis, one should keep in mind that phylogenetic analyses of any single genetic marker may result in a phylogeny that is inconsistent with the evolutionary history of the species (Pamilo and Nei, 1988). This has been evident e.g. in the co-divergence study between genetic lineages of PUUV and bank voles (Nemirov et al., 2010). Here, the analysis of SWSV co-evolution with its host is complicated by the fact that the CRs and mitochondrial phylogeography in *S. araneus* are not congruent (Lundqvist et al., 2011; Shchipanov and Pavlova, 2017). Based on a recent study suggesting that karyotypic changes reflect well the evolutionary relationships among *Sorex* species (Mackiewicz et al., 2017), we decided to use the CRs for our analyses. In this study, admittedly, the CRs of the our common shrews are not confirmed by laboratory tests. However, the CRs and their ranges in Fennoscandia have been examined carefully in earlier studies (Wójcik et al., 2003). Further inconsistencies rise from the observed reassortment events of viral genomes. In the southern, central, and eastern populations of Finnish *S. araneus*, SWSV reassortment events were detected, suggesting the co-circulation of different lineages (FIN-2 and FIN-3) possibly at the hybrid zones between CRs. CRs are essentially nonoverlapping, and hybrid zones between chromosomal karyotypes are narrow, usually only some kilometers (Andersson et al., 2004), but apparently enough to support reassortation. Recombination has not been detected in SWSV yet, however, homologous recombination between strains of PUUV has been found and even regarded as the reason for contradictory results from the S- and M- phylogeny (Nemirov et al., 2010). Ancient recombination events might also have happened in other insectivore-borne hantaviruses (Kang et al., 2009b).

Our large data set of new SWSV sequences allowed for phylogeographic analyses of SWSV in Finland, despite all the complications discussed above. We suggest that the postglacial spread of SWSV into Finland mirrors that of the host, *S. araneus*, at least on the basis of the S-segment and chromosomal race evolution of the host. The phylogeographic structure of SWSV is interesting because it reflects the chromosomal evolution of the host very recently, during the last 10,000 years. The results show that the virus is similar to rodent-borne hantaviruses as it seems to have dispersed with the host migrations. The incongruence of S- and L- phylogeny once again suggests that hantavirus evolution is more complicated than we expected.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.meegid.2017.11.010.

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