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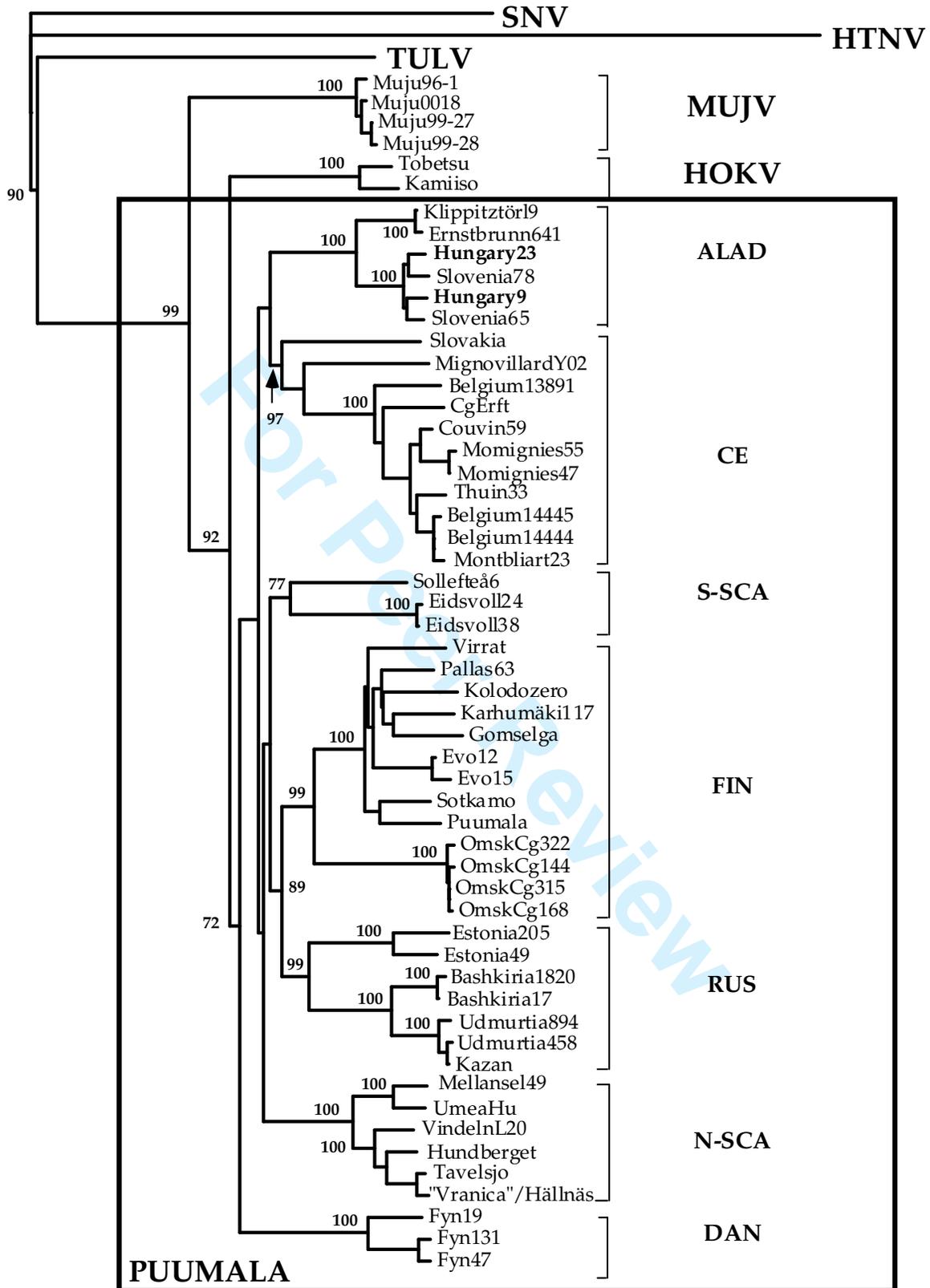
Co-circulation of three pathogenic hantaviruses: Puumala, Dobrava and Saaremaa in Hungary

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Keywords:	HFRS; hantavirus; Puumala virus, Dobrava virus, Saaremaa virus

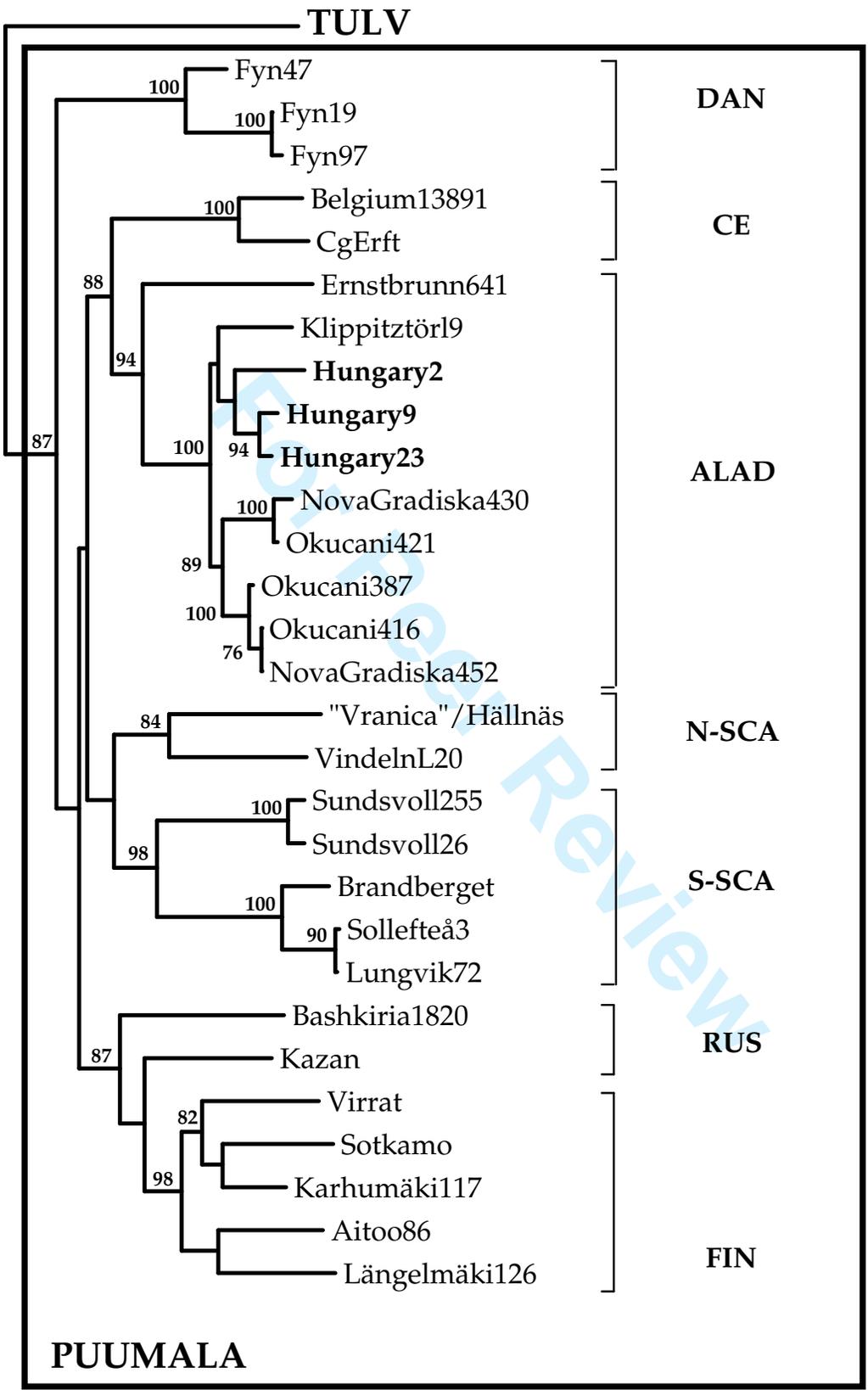


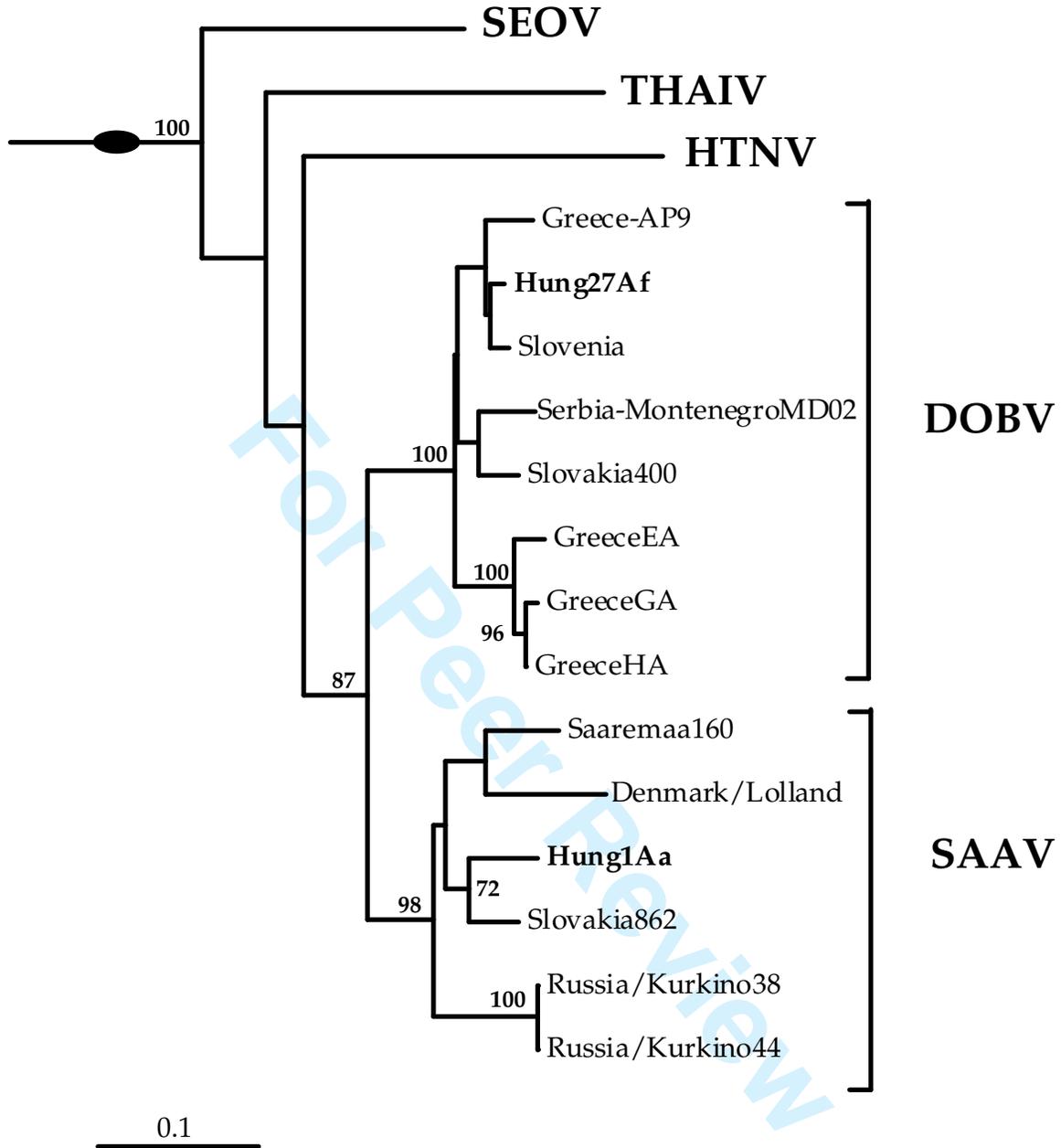


Fig. 1. Rodent collecting localities in Hungary (shown as full circles). Two localities (TR16 and TR17) that are discussed in the article are marked. Stars indicate localities from where hantavirus sequences have been recovered earlier (Scharninghausen et al., 1999; Jakab et al., 2007a, 2008).
540x414mm (72 x 72 DPI)

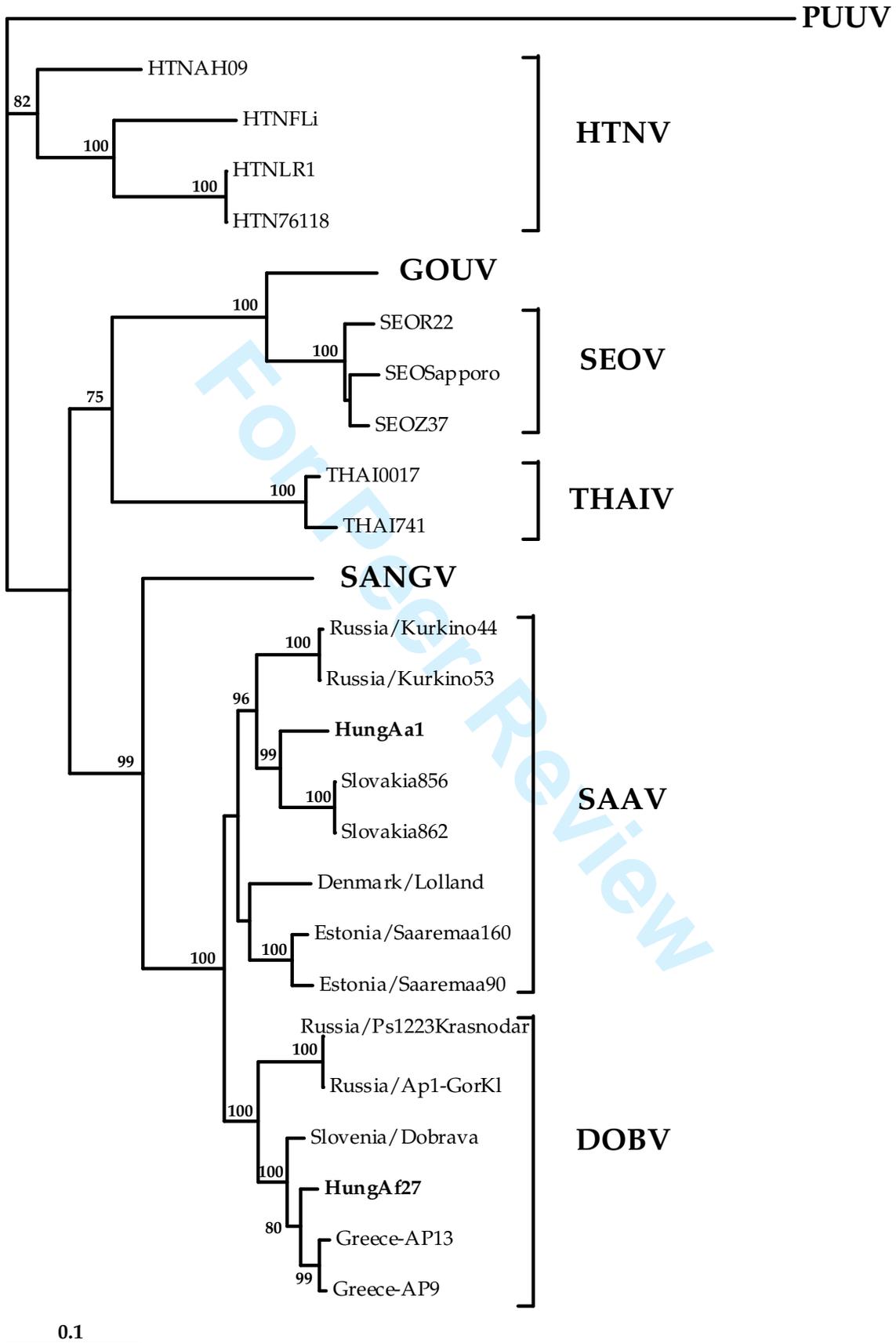


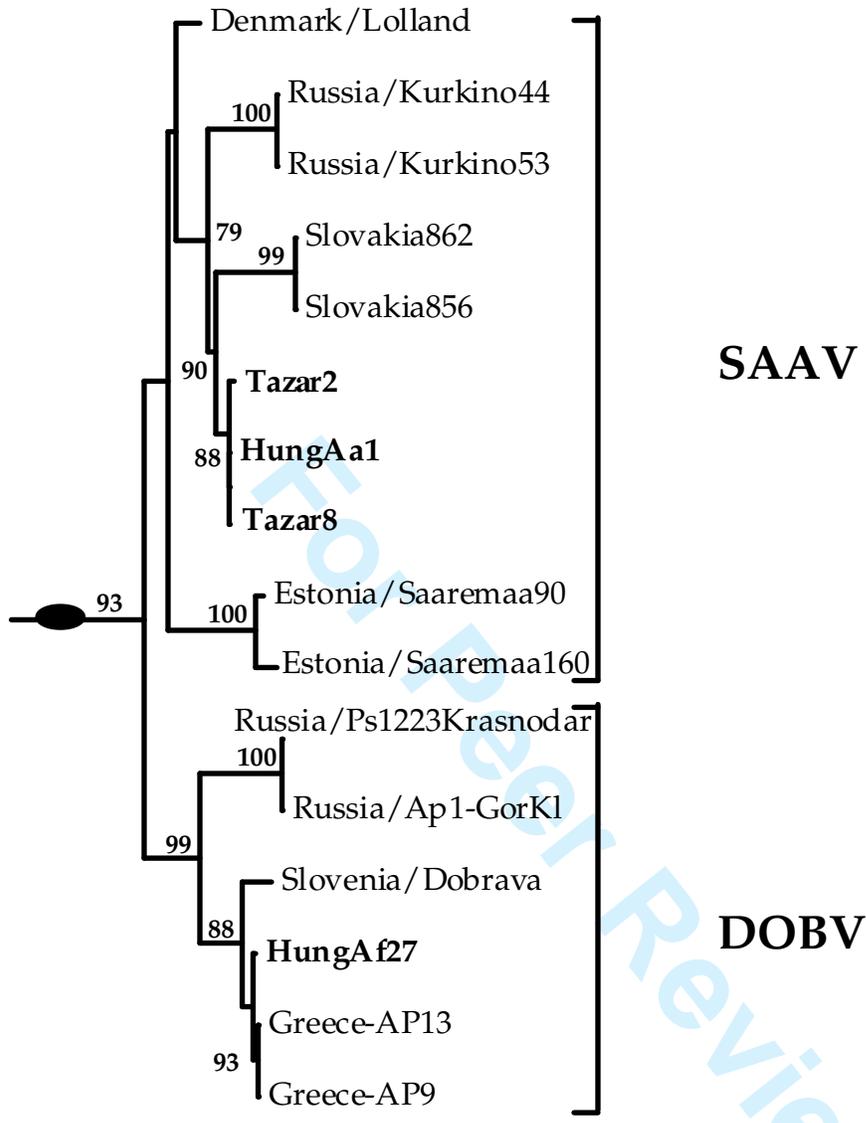
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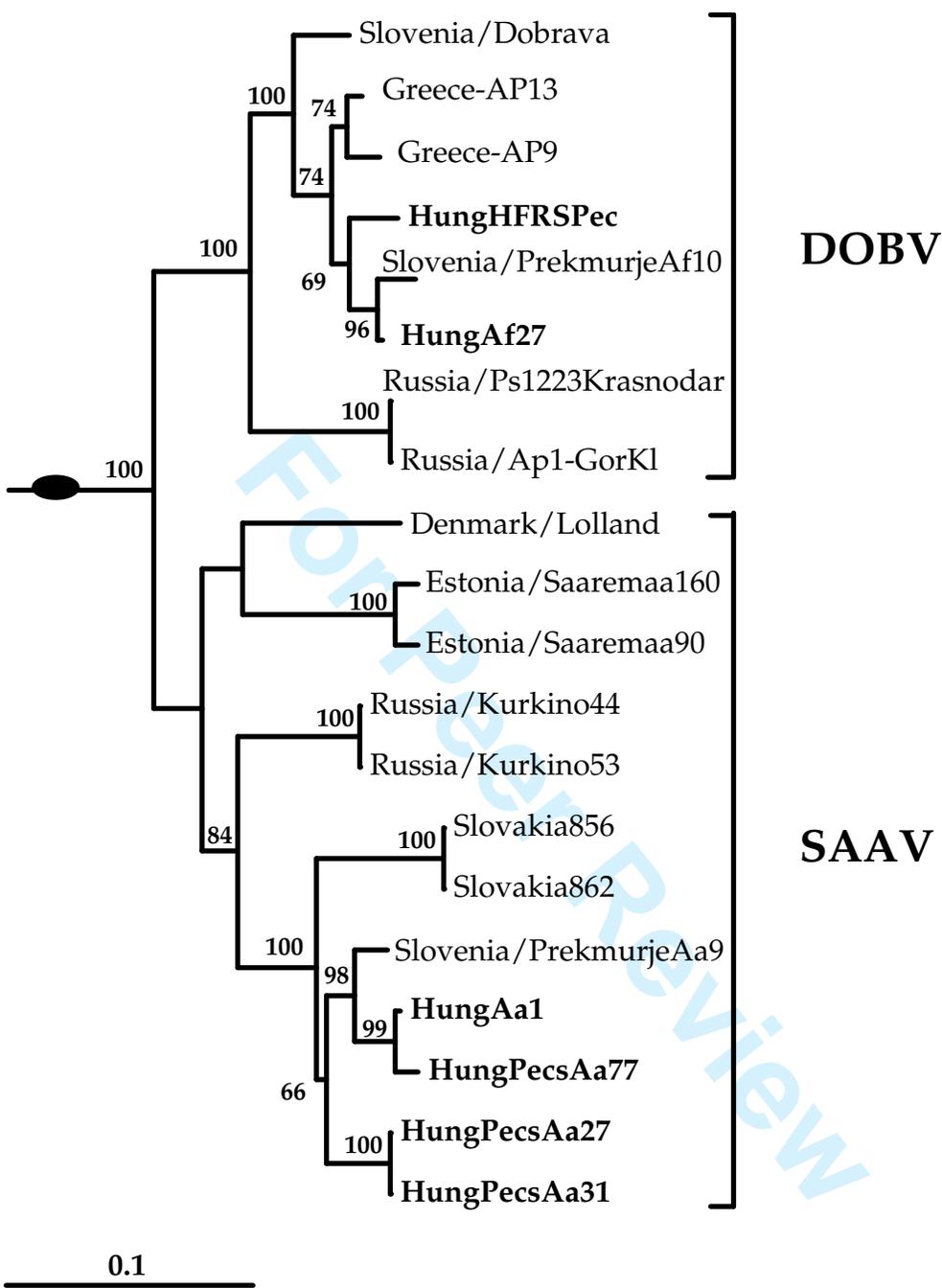
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29 **ABSTRACT**

31 Hantaviruses (*Bunyaviridae*) cause hemorrhagic fever with renal syndrome (HFRS) in
32 Eurasia and hantavirus (cardio)pulmonary syndrome (HCPS) in the Americas. HFRS
33 is caused by Hantaan (HTNV), Seoul (SEOV), Dobrava (DOBV), Saaremaa (SAAV)
34 and Puumala (PUUV) viruses. Of those, only HTNV is not present in Europe. In
35 recent years, hantaviruses, described in other parts of Europe, were also detected at
36 various locations in Hungary. To study the genetic properties of Hungarian
37 hantaviruses in detail, sequences of the viral S and M segments were recovered from
38 bank voles (*Myodes glareolus*), yellow-necked mice (*Apodemus flavicollis*) and striped
39 field mice (*Apodemus agrarius*) trapped in the Transdanubian region. As expected, the
40 sequences recovered belonged, respectively, to PUUV (two strains), DOBV (one
41 strain) and SAAV (one strain). On phylogenetic trees two new Hungarian PUUV
42 strains located within the well-supported Alpe-Adrian (ALAD) genetic lineage that
43 included also Austrian, Slovenian and Croatian strains. Analysis of the Hungarian
44 SAAV and DOBV genetic variants showed host-specific clustering and also
45 geographical clustering within each of these hantavirus species. Hungarian SAAV
46 and DOBV strains were related most closely to strains from Slovenia (Prekmurje
47 region). This study confirms that multiple hantaviruses can co-circulate in the same
48 locality and can be maintained side-by-side in different rodent species.

51 **Key words:** HFRS; hantavirus; Puumala virus, Dobrava virus, Saaremaa virus.

1
2 52 **INTRODUCTION**
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7 Hantaviruses (genus *Hantavirus*, family *Bunyaviridae*) cause hemorrhagic fever
8 with renal syndrome (HFRS) in Eurasia and hantavirus (cardio)pulmonary
9 syndrome (HCPS) in the Americas (for reviews, see Schmaljohn and Hjelle, 1997;
10 Vapalahti et al., 2003). HFRS is caused by *Hantaan virus* (HTNV), *Seoul virus* (SEOV),
11 *Dobrava-Belgrade virus* (DOBV), *Saaremaa virus* (SAAV) and *Puumala virus* (PUUV).
12 *Sin Nombre virus* (SNV), *Andes virus* and related hantaviruses are the main causative
13 agents of severe HCPS. Hantaviruses are host-specific, and thus their distribution
14 correlates most often with the geographical distribution of the natural host species,
15 rodents and insectivores. Transmission of hantaviruses to humans occurs by
16 inhalation of infested aerosols of rodent excreta. Whether hantaviruses carried by
17 insectivores can infect humans and cause any disease remains to be seen.
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29 65 Of HFRS-causing hantaviruses, only HTNV is not present in Europe [Vapalahti
30 et al., 2003]. PUUV, carried by bank voles (*Myodes glareolus*), is the most widely
31 distributed European hantavirus. It causes a mild form of HFRS (also called
32 *nephropathia epidemica*, NE) in Northern and Central Europe, European part of Russia
33 and in the Alpe-Adrian region. DOBV, harbored by yellow-necked field mice
34 (*Apodemus flavicollis*), is the most severe European hantavirus pathogen; it is
35 associated with HFRS mostly in the Balkans [Papa et al., 1998; Avsic-Zupanc et al.,
36 1999]. SAAV, carried by striped field mice (*Apodemus agrarius*), causes mild HFRS
37 resembling NE; the virus is found in the Baltics, Central Europe and European part
38 of Russia [Lundkvist et al., 1998; Plyusnin et al., 1999; Golovljova et al., 2000; Sibold
39 et al., 2001]. SEOV, carried by Norway rats (*Rattus norvegicus*), is so far found in
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2 76 France and Belgium [Heyman et al., 2004, 2009]. Apart from laboratory outbreaks
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4 77 caused by SEOV-infected rats kept in captivity, no human cases in Europe have
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6 78 been reported in connection to this hantavirus.
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9 79 HFRS has been described in Hungary prior to the identification of the
10
11 80 causative agents, the hantaviruses [Trencsenyi and Keleti, 1981]. Until 1985, human
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13 81 HFRS cases were diagnosed initially based on medical symptoms and
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15 82 epidemiology; these were later confirmed using serological tests [Takenaka et al.,
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17 83 1985; Faludi and Ferenczi 1995]. With the advent of DNA and RNA sequencing
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19 84 several hantaviruses have been identified in Hungary [Scharninghausen, 1999,
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21 85 Ferenczi 2003, Jakab 2007a, 2008].
22

23 86 On average, there are 20-25 laboratory-confirmed human hantavirus infections
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25 87 in Hungary each year. The fatality rate associated with clinical infections dropped
26
27 88 from approximately 6% in the 1950s to 2.5% in recent years. In case a physician
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29 89 suspects HFRS, further serological tests are performed at the National Center for
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31 90 Epidemiology (NCE). Serological diagnosis is based primarily on
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33 91 immunofluorescence assay (IFA) and secondarily on enzyme-immunoassay (EIA).
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35 92 Compared to the number of hospitalized patients, hantavirus seroprevalence is
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37 93 much higher in Hungary, approximately 10% [Ferenczi et al., 2005]. These
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39 94 controversial data might be explained by the low HFRS awareness of the medical
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41 95 community and by the undiagnosed mild forms of the disease.
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43
44 96 The aim of this study was to recover hantavirus sequences from tissue samples
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46 97 of rodents known as natural hosts for Puumala, Dobrava and Saaremaa viruses,
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48 98 perform their detailed (phylo)genetic characterization, assign them to proper taxa
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50 99 and specify unequivocally the rodent hosts involved.
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MATERIALS AND METHODS

Trapping of rodents. Small mammals were collected as part of a larger research project on studying the distribution of hantaviruses in Hungary. The broader area, the southwestern part of Hungary, was selected based on the high incidence rate of human hantavirus cases (Fig. 1). Zala county with only 3% of Hungary's total population gives 23% of HFRS cases in the country. In 2000, selected trapping localities were chosen to survey wild mammals as reservoirs of hantaviruses in forest habitats. The primary factor in selecting specific sites was the forest area, but several other characteristics of the plant community (e.g. disturbance level and plant species composition) correlated with patch size. Rodents were trapped using Sherman type live capture traps [Mills et al., 1995] set in the evening and checked in the morning. At each locality trapping was continued until sufficient numbers of animals (usually around 40-50) were captured. Animals were identified, aged, sexed and their conditions were recorded. Euthanized rodents were dissected; blood and tissue samples – lung, spleen, kidney, heart – were removed for further analysis. Tissues were frozen immediately in liquid nitrogen. Upon arrival at the Virology Department of the NCE tissue samples were transferred to -80°C freezers for long-term storage. Skeletal materials of the collected animals were deposited as voucher specimens at the Mammal Collection of the Museum of Southwestern Biology, University of New Mexico.

Screening of rodent samples. Sera collected from rodents were screened initially for the presence of antibodies to hantaviruses by commercially available kits as described previously [Ferenczi et al., 2003]. Briefly, HTNV antigen-coated

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2 125 high density particle agglutination (HDPA) kit (Korean Green Cross) was used
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4 126 according the protocol of the WHO Collaborating Center for Virus Reference and
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6 127 Research (HFRS), Institute for Viral Diseases, Korea University, Seoul, South Korea.
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8 128 To perform indirect immunofluorescent assay (IFA), HTNV-, PUUV- and SEOV-
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10 129 specific tests (Progen Biotechnik, GMBH, Germany) with FITC-conjugated rabbit
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12 130 anti-mouse immunoglobulin (DAKO, A/S, Denmark) were used. For the detection
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14 131 of HTNV- and PUUV-specific antibodies by enzyme immunoassay (EIA), IgG and
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16 132 IgM kits (Progen Biotechnik) were applied.

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19 133 Twenty five out of 37 serum samples were tested additionally on EIA plates
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21 134 containing recombinant antigens for PUUV, DOBV and SEOV viruses [Elgh et al.,
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23 135 1997]. Lung tissue samples from seropositive rodents were screened further for
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25 136 hantavirus nucleocapsid (N) protein antigen by immunoblotting as described earlier
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27 137 [Plyusnin et al., 1995].

30 138 **Reverse transcription - polymerase chain reaction (RT-PCR) and sequencing.**

31 139 RNA was extracted from lung tissue samples using the Tripure reagent (Boehringer
32
33 140 Mannheim) following recommendations of the manufacturer. RNA was then
34
35 141 subjected to RT-PCR to recover partial S segment and M segment sequences
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37 142 (sequences of primers and other experimental details are available upon request).
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39 143 PCR amplicons were gel-purified with QIAquick Gel Extraction -kit (QIAGEN) and
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41 144 sequenced directly using ABI PRISM™ Dye Terminator sequencing kit (Perkin
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43 145 Elmer/ABI, NJ). PUUV genome sequences described in this paper have been
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45 146 deposited to the GenBank sequences database under accession numbers FN377821-
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47 147 25. DOBV and SAAV genome sequences described in this paper have been deposited
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49 148 to the GenBank sequences database under accession numbers FN377826-29.
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149 **Mitochondrial DNA (mtDNA) analysis** was performed as described earlier
150 [Nemirov et al., 2002]. Briefly, DNA was extracted from lung tissue samples using
151 the Tripure reagent. A 427 nt-long PCR-product from the D-loop-encoding region
152 was amplified with primers 5'-CCACCATCAGCACCCAAAGCTG-3' and 5'-
153 CTGAAGTAAGAACCAGATGTCTG-3'. The product was purified from the gel and
154 subjected to direct sequencing. For comparison, mtDNA sequences of *A. agrarius* and
155 *A. flavicollis* were retrieved from the GenBank nucleotide databases.

156 **Phylogenetic analysis.** Multiple nucleotide alignments were prepared
157 manually using SeqApp 1.9a169 sequence editing program. Phylogenetic analysis
158 was performed using the PHYLIP program package [Felsenstein, 1993]. 500
159 bootstrap replicates (Seqboot program) were submitted to the distance matrix
160 algorithm (Dnadist program), with maximum likelihood model for nucleotide
161 substitutions; distance matrices were analysed with the Fitch-Margoliash tree-fitting
162 algorithm (Fitch program); the bootstrap support values were calculated with the
163 Consense program. Hantavirus sequences used for comparison were recovered from
164 the GenBank.

RESULTS**Prescreening of rodents.** Rodent candidates for further RT-PCR and

sequencing were pre-screened for hantavirus antibodies by HDPa, IFA and EIA.

From more than 300 rodents, 37 were found positive or suspected positive: 8 bank voles (*Myodes glareolus*) and 29 *Apodemus* mice (18 *A. agrarius*, 2 *A. flavicollis*, 9 - unspecified *Apodemus* spp.). Positive *M. glareolus* were from the locality TR17, positive *Apodemus* mice from localities TR16 and TR17 (Fig. 1).

Genetic analysis of PUUV strains. From eight pre-selected (Ab-positive) bank

voles, six were found positive for PUUV N-Ag in immunoblotting. Of those, three

appeared also positive for PUUV genome when tested by RT-PCR; all three animals

were from the same locality, TR-17. Three wild-type PUUV strains (that were not

isolated) were designated PUUV/Mg2/HungaryTR17/00,

PUUV/Mg9/HungaryTR17/00, and PUUV/Mg23/HungaryTR17/00, or Hung2,

Hung9, and Hung23, for short. For two strains, Hung9 and Hung23, almost

complete sequences of the S segment have been recovered in a set of (semi)-nested

PCRs. For the strain Hung2, only partial sequence of the coding region (nt 191 to

1190) was recovered. It differed by two silent nucleotide substitutions from the

corresponding sequence of strain Hung9 and was excluded from further analyses.

Two other sequences included 5'-noncoding region (NCR) of 42 nt and the open

reading frame (ORF) for the N protein of 433 aa followed by 499 or 442 nt from 3'-

NCR (for strains Hung9 and Hung23, respectively). The 5'-NCR sequences were

identical (the first 22 nt originated from the primer sequence and thus were not

determined directly); the coding regions differed by 34 substitutions (identity of

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2 189 97.4%); the 3'-NCR sequences differed at 28 positions (identity of 95.9%). Deduced N
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4 190 protein sequences differed by one substitution only (identity of 99.8%) and carried
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6 191 signature residues V236 and P257 shared also by PUUV strains from neighbouring
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8 192 Austria [Plyusnina et al., 2006] and Slovenia [Avsic-Zupanc et al., 2007]. Partial M
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10 193 segment sequences (nt 2161-2570, primers excluded) of strains Hung2, Hung9, and
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12 194 Hung23 were recovered as well; they appeared 94.4-96.1% identical to each other.
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14 195 The encoded partial sequences of the Gc protein (136 aa long) were 97.8-99.3%
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16 196 identical. Hungarian M segment/Gc protein sequences were most closely related to
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18 197 the corresponding sequences from Austrian [Plyusnina et al., 2006] and Croatian
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20 198 [Cvetko et al., 2005] PUUV strains (corresponding sequences from Slovenian strains
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22 199 are not available).

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25 200 On the phylogenetic tree based on the complete S segment coding region, the
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27 201 two Hungarian PUUV strains were located within the well-supported Alpe-Adrian
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29 202 (ALAD) genetic lineage that included also Austrian and Slovenian strains (Fig. 2A).
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31 203 Within this lineage, Hungarian and Slovenian strains shared the most recent
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33 204 common ancestor (TMRCA) while Austrian strains formed a sister taxon to this
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35 205 quartet. Six other PUUV lineages can be recognized: (1) Central European (CE); (2)
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37 206 South Scandinavian (S-SCA); (3) Finnish (FIN); (4) Russian (RUS); (5) North
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39 207 Scandinavian (N-SCA); and (6) Danish (DAN). PUUV shared more ancient common
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41 208 ancestors (MACAs) with Hokkaido virus (HOKV) and then with Muju virus
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43 209 (MUJV) associated with *Myodes rufocanus* and *Myodes regulus*, respectively. The
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45 210 phylogenetic tree based on the partial M segment sequence showed similar topology
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48 211 (Fig. 2B): Hungarian strains were located within the ALAD lineage which, in this
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2 212 case, included also strains from Austria and Croatia. Other PUUV lineages (CE, S-
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4 213 SCA, FIN, RUS, N-SCA and DAN) were presented as well.

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6 214 **Genetic analysis of SAAV and DOBV strains.** From 29 pre-screened
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8 215 *Apodemus* mice (18 *A. agrarius*, 2 *A. flavicollis*, 9 - unspecified); six were found
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10 216 positive for DOBV/SAAV N-Ag in immunoblotting. Of those, only two were
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12 217 positive also in the RT-PCR test. Analysis of mtDNA sequences showed that one
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14 218 mouse (#1, from locality TR-16) belonged to the species *A. agrarius*, another (#27
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16 219 from locality TR-17) was *A. flavicollis*. Partial S and M segment sequences were
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18 220 recovered from both rodents using DOBV/SAAV-specific primers and subjected to
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20 221 phylogenetic analysis. On phylogenetic trees (Fig. 3A-B), the sequence recovered
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22 222 from *A. agrarius* mouse was placed with SAAV sequences (all recovered from
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24 223 striped field mice) while the sequence recovered from *A. flavicollis* mouse co-
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26 224 localized with DOBV sequences, originated from either yellow-necked mice or
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28 225 human HFERS cases. Although the topologies of the trees based on partial M and S
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30 226 segment sequences were quite similar, the M-tree showed a somewhat better
31
32 227 resolution, i.e. higher bootstrap support values for SAAV- and DOBV-clades. Based
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34 228 on these data, novel Hungarian hantavirus strains were designated
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36 229 SAAV/Aa1/Hungary TR16/00 and DOBV/Af27/HungaryTR17/00 or HungAa1
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38 230 and HungAf27, for short.

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41 231 New Hungarian SAAV and DOBV sequences were compared to previously
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43 232 published ones recovered from *A. agrarius* trapped in Taszár [spelled as "Tazar" in
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45 233 the original publication], eastern Hungary [Scharninghausen et al., 1999]. The
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47 234 analysis was restricted to the overlapping part of the S segment sequence recovered
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49 235 in two studies: nt 707 to 935. This region of two Taszár strains appeared most
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2 236 closely related to the corresponding region of the SAAV strain HungAa1: only one
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4 237 (silent) nucleotide substitution was observed between these sequences (identity of
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6 238 99,6%). In contrast, the sequence of the DOBV strain HungAf27 differed from Taszár
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8 239 sequences at 29-30 positions (identity of 86.9-87.3%). Phylogenetic analysis showed
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10 240 TMRCA for the two Taszár strains and the strain HungAa1 (Fig. 3C). This trio was
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12 241 placed within the SAAV clade and shared MACA with SAAV strains from Slovakia.
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14 242 These data suggested that the two Taszár strains belong to SAAV.

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17 243 New Hungarian SAAV and DOBV sequences were compared also to the
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19 244 previously published sequences recovered from *A. agrarius* trapped in the Görcsöny
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21 245 and Sármellék areas and from an HFRS patient from the University hospital of Pécs
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23 246 (all in the Transdanubian region) [Jakab et al., 2007a,b]. Since, in this case, the
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25 247 analysis was restricted to shorter sequences of the S segment recovered by these
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27 248 authors (nt 410 to 800), it became possible to include also partial SAAV and DOBV
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29 249 sequences originated from Prekmurje region of neighbouring Slovenia [Avsic-
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31 250 Zupanc et al., 2000].

32
33 251 Direct sequence comparison showed that Hungarian SAAV strain HungAa1
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35 252 was most closely related to Hungarian strain Pecs/77Aa from Sármellék (sequence
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37 253 identity 99.5%) and to Slovenian SAAV strain Aa9 from Prekmurje (97.9%).
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39 254 Sequence of our DOBV strain HungAf27 was most closely related to the
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41 255 corresponding region of Slovenian DOBV strain from Prekmurje (99.2%) and then to
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43 256 Hungarian HFRS-associated strain (92.0%). On the phylogenetic tree (Fig. 3D) all
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45 257 four Hungarian *A. agrarius* originated sequences, as expected, were located within
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47 258 the SAAV clade. The new strain HungAa1 shared TMRCA with the strain PecsAa77
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49 259 and this couple shared the MACA with Slovenian strain Aa9 from Prekmurje.
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2 260 Similarly, our DOBV strain HungAf27 shared TMRCA with Slovenian DOBV strain
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4 261 Af10 from Prekmurje; they, in turn, shared MACA with the Hungarian HFRS-
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6 262 associated strain. These results demonstrated host-specific clustering of Hungarian
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8 263 SAAV and DOBV genetic variants and, within each of these hantavirus species, also
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10 264 geographical clustering.
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DISCUSSION

The results of this study demonstrate co-circulation of all three major European hantavirus pathogens in Hungary: PUUV, DOBV and SAAV. They are in line with earlier observations on human HFRS cases in this country [Ferenczi et al., 2005; Jakab et al., 2007a] and recovery of hantavirus sequences from rodents [Scharninghausen et al., 1999; Ferenczi et al., 2003; Jakab et al., 2007a]. It should be emphasized that while SAAV in Hungarian striped field mice has been already reported (albeit under the name of Dobrava virus - see below), the first unequivocal evidence for PUUV and DOBV in Hungarian rodents, species *M. glareolus* and *A. flavicollis*, respectively, is presented in this paper.

Rodents used in genetic studies were selected in two steps. First, pre-selection by HDPA, EIA and IFA to detect hantavirus antibodies was performed. On that stage, not only clearly Ab-positive but also borderline voles and mice were selected for further analyses. This could explain the observed differences between the results of antibody detection and the N-Ag detection, due to pre-selection not only clearly Ab-positive but also voles and mice giving the borderline results. In turn, the relatively low RT-PCR positivity of N-Ag-positive rodents could be due to partial degradation of target RNA in tissue samples that were subjected to several thawing-freezing cycles (to take material for other analyses) before RNA extraction.

Phylogenetic analysis of Hungarian PUUV strains placed them within the ALAD genetic lineage that includes also strains from neighbouring Slovenia, Croatia and Austria. This presents a good example of geographical clustering of PUUV genetic variants, a phenomenon first discovered more than a decade ago [Plyusnin

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2 289 et al., 1994]. Phylogenies that included new Hungarian PUUV sequences confirmed
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4 290 two interesting earlier observations [Plyusnina et al., 2006]. First, within the ALAD
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6 291 lineage, two Austrian M-sequences are not monophyletic. One of the strains, Klipp9,
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8 292 is clustering together with Croatian and Hungarian strains suggesting that M and S
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10 293 segments of this strain might have different evolution history, i.e. this strain is a
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12 294 reassortant one. Second, ALAD and CE lineages and RUS and FIN lineages share
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14 295 MACAs thus suggesting somewhat closer relationships within these two pairs of
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16 296 lineages that could be rooted to the last postglacial recolonization of these areas of
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19 297 Europe.

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21 298 The analyses of Hungarian SAAV and DOBV sequences confirmed that genetic
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23 299 variants of these hantavirus species show the host-specific clustering. Indeed, all
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25 300 sequences recovered from *A. agrarius* belonged to SAAV while those from *A.*
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27 301 *flavicollis* belonged to DOBV. Thus previously reported sequences from Hungarian
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29 302 *A. agrarius* [Scharninghausen et al., 1999; Jakab et al., 2007a] should be re-classified
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31 303 as SAAV strains. It seems that only the sequence recovered from human HFERS case
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33 304 in Pécs [Jakab et al., 2007b] presents *bona fide* DOBV. As mentioned above, for
34
35 305 several years there was a controversy in taxonomy and hence terminology of SAAV.
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37 306 When first discovered, this Dobrava-like virus was erroneously considered a genetic
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39 307 variant of DOBV carried by the striped field mice [Plyusnin et al., 1997; Nemirov et
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41 308 al., 1999]. It took several years to realize that this is in fact a distinct hantavirus
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43 309 species [Brus-Sjölander et al., 2002; Nemirov et al., 2002], the view that is now
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45 310 shared by the International Committee for Virus Taxonomy [www.ictvonline.org].
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48 311 Another important finding of this study is that multiple hantavirus types can
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50 312 be maintained at the same locality by their corresponding rodent hosts. The direct
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2 313 transmission mode and the high level of host specificity of the hantaviruses make
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4 314 these temperate-zone viruses less affected by host biodiversity [Ruedas et al., 2004].
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6 315 Instead, the population density and size are the main factors driving the disease
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8 316 temporal dynamics.

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10 317 Of the four European hantavirus pathogens known so far, only SEOV has not
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12 318 been found in Hungary, albeit serological evidence showed its causative role at least
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14 319 in one case [Ferenczi, unpublished data]. Direct detection of this virus is rather
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16 320 difficult because its primary hosts, Norway rats *Rattus norvegicus*, would not be
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18 321 caught by the traps used. Similar observation have been made in neighbouring
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20 322 Slovenia [Avsic-Zupanc et al., 2002, 2007], Slovakia [Sibold et al., 2001] and Croatia
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22 323 [Cvetko et al., 2004]. Our unpublished serological data on clinical cases confirm that
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24 324 both PUUV-like and DOBV/SAAV-like HFRS cases occur in Hungary. Co-
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26 325 circulation of three distinct hantaviruses that could cause HFRS of different severity,
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28 326 including life-threatening infections, calls for improvements in both surveillance
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30 327 and diagnostics.

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2 436 **Legends to figures**
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5 438 **Fig. 1.** Rodent collecting localities in Hungary (shown as full circles). Two
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7 439 localities (TR16 and TR17) that are discussed in the article are marked. Stars indicate
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9 440 localities from where hantavirus sequences have been recovered earlier
10 441 (Scharninghausen et al., 1999; Jakab et al., 2007a, 2008).
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12 442
13 443 **Fig. 2.** Phylogenetic trees of Puumala and related hantaviruses based on (A)
14 444 complete S segment coding sequences and (B) partial M segment sequences (nt 2161-
15 445 2570). Numbers show the bootstrap support values for the branching points (500
16 446 replicates). Only the values higher than 70% are shown. Abbreviations: **SNV**, Sin
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18 447 Nombre virus, strain NM H10; **HTNV**, Hantaan virus, strain 76-118; **TULV**, Tula virus,
19 448 strain Moravia02v; **MUJV**, Muju virus; **HOKV**, Hokkaido virus. Genetic lineages of
20 449 PUUV: **ALAD**, Alpe-Adrian; **CE**, Central European; **S-SCA**, South Scandinavian; **FIN**,
21 450 Finnish; **RUS**, Russian; **N-SCA**, North Scandinavian; **DAN**, Danish.
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27 452 **Fig. 3.** Phylogenetic trees of Dobrava, Saaremaa and related hantaviruses based on
28 453 (A) partial M segment sequences (nt 1704-1969), and partial S segment sequences: (B) nt
29 454 377-935; (C) nt 707-935; (D) nt 410-800. Numbers show the bootstrap support values for
30 455 the branching points (500 replicates). Only the values higher than 50% are shown.
31 456 Abbreviations: **DOBV**, Dobrava virus; **SAAV**, Saaremaa virus; **HTNV**, Hantaan virus,
32 457 strain 76-118 (if not specified otherwise); **SEOV**, Seoul virus, strain Sapporo rat (if not
33 458 specified otherwise); **THAIV**, Thailand virus, strain741; **GOUV**, Gou virus, strain
34 459 Nc167; **SANGV**, Sangassou virus strain SA14; **PUUV**, Puumala virus, strain Sotkamo.
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