

**Microheterogeneity  
of the Volgograd  
clone  
of West Nile virus  
and its endemicity**



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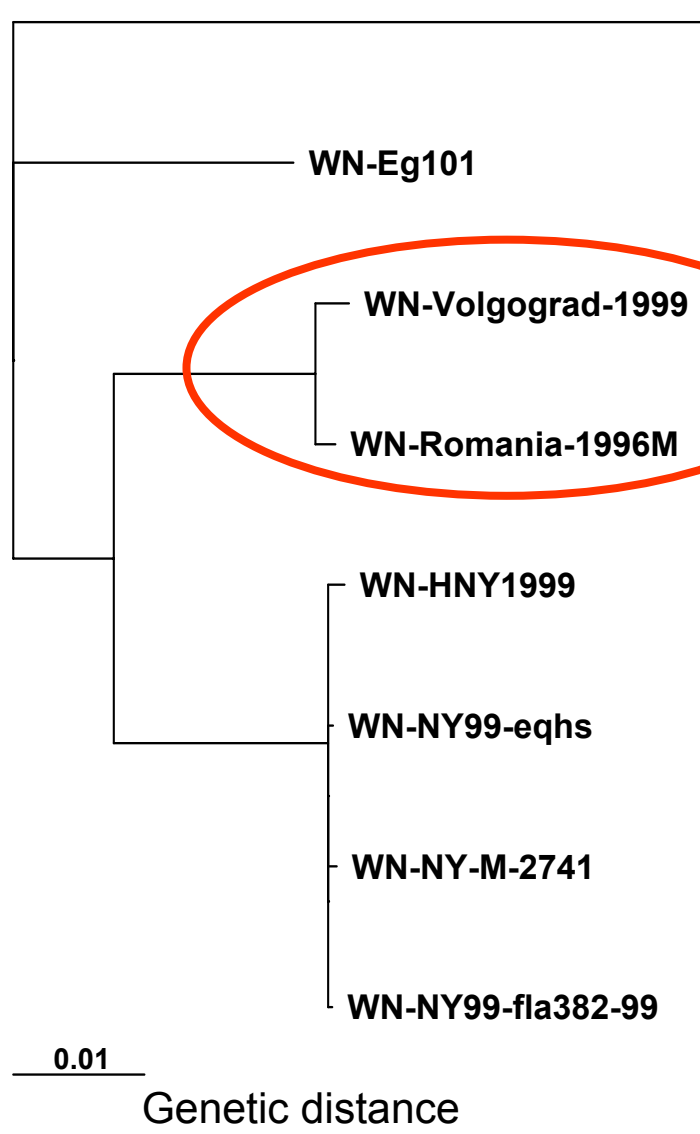
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# West Nile fever in Russia 1999 - affected regions



Three regions have reported WN cases:  
Volgograd (> 500 cases, 40 deaths);  
Astrakhan (> 90 cases, 5 deaths);  
Krasnodar (> 40 cases, 3 deaths).

# Genetic distance of West Nile virus isolates based on complete nucleotide sequence



Kunjin-MRM61C

Similar strains:

1. "New York group" – equine, flamingo, human, and mosquito isolates in 1999.
2. Mosquito isolate, Romania 1996 and human clinical isolate, Volgograd 1999.

OTUs	штамм	1	2	3	4	5	6	7	8
1	WN-Volgograd-1999		0.42	4.49	3.51	3.55	3.48	3.61	13.35
2	WN-Romania-1996M	0.06		4.38	3.35	3.40	3.34	3.46	13.38
3	WN-Eg101	0.20	0.19		4.52	4.56	4.49	4.62	12.86
4	WN-NY99-eqhs	0.18	0.17	0.20		0.11	0.08	0.16	13.22
5	WN-MNY-2741	0.18	0.17	0.20	0.03		0.12	0.20	13.22
6	WN-NY99-F382-99	0.18	0.17	0.20	0.03	0.03		0.17	13.20
7	WN-HNY1999	0.18	0.17	0.20	0.04	0.04	0.04		13.25
8	Kunjin-MRM61C	0.33	0.33	0.32	0.33	0.33	0.33	0.33	

Percentage of nucleotide difference (upper row) and standard error (lower row), MEGA



The nearest relative of the Volgograd WN isolate was the mosquito isolate from Romania-1996. The strains differed from each other in 46 nucleotide positions, 43 of them were located in coding region. 35 mutations were silent so the strains differed in 8 amino acid only. So the homology of WN-Volgograd-4 and WN-Romania-1996M strains was 99.77% for aa sequence and 99.58% for nt sequence.

**Is the difference of 0.42% really small?**

**★ Do these strains belong to the same clone?**



**What is a West Nile virus clone?**

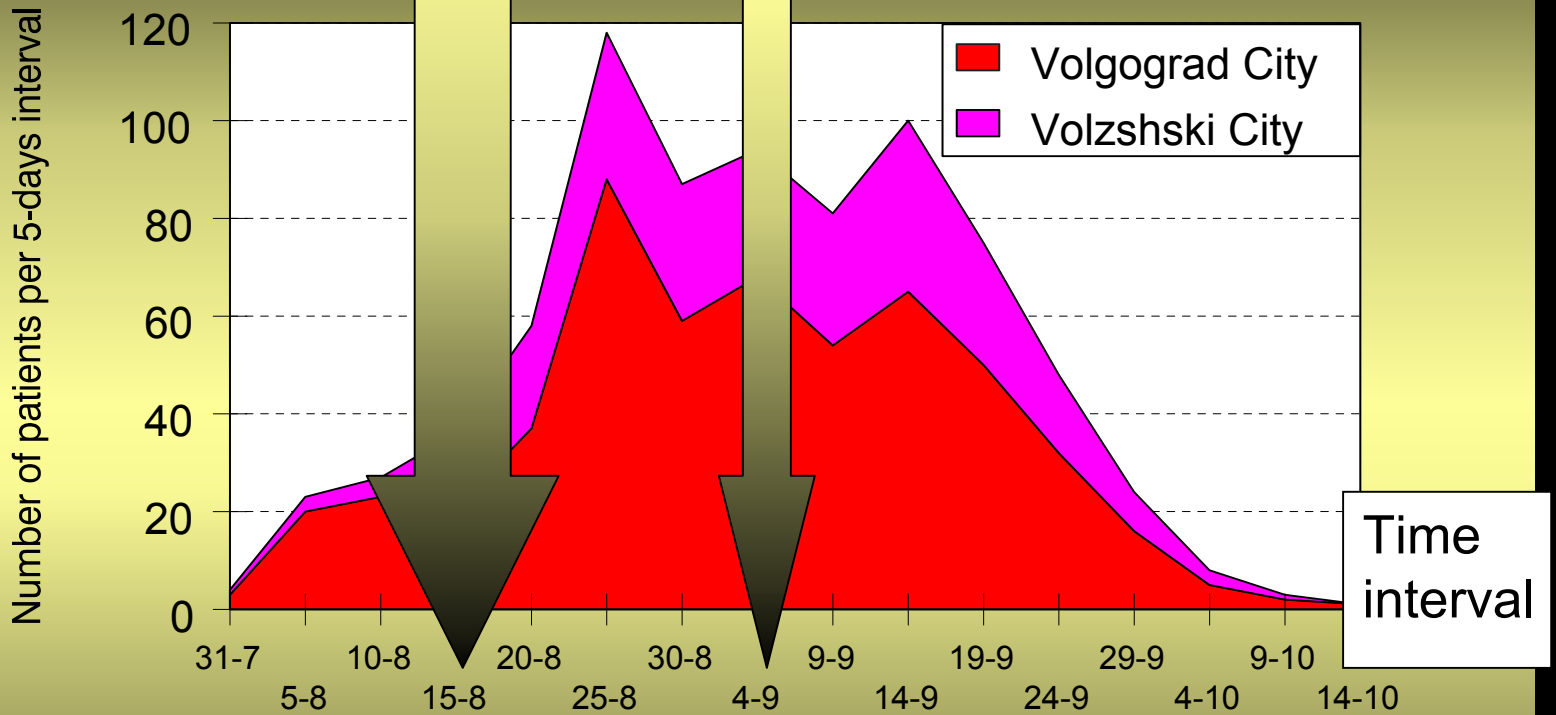
**If the isolates are obtained from the same hosts (humans) who contracted the disease at the same time and at the same place, these isolates appeared belonging to the same clone.**

**Minor differences might arise as a result of "genetic noise" or adaptation to host or vector.**



**Period of patient hospitalization**

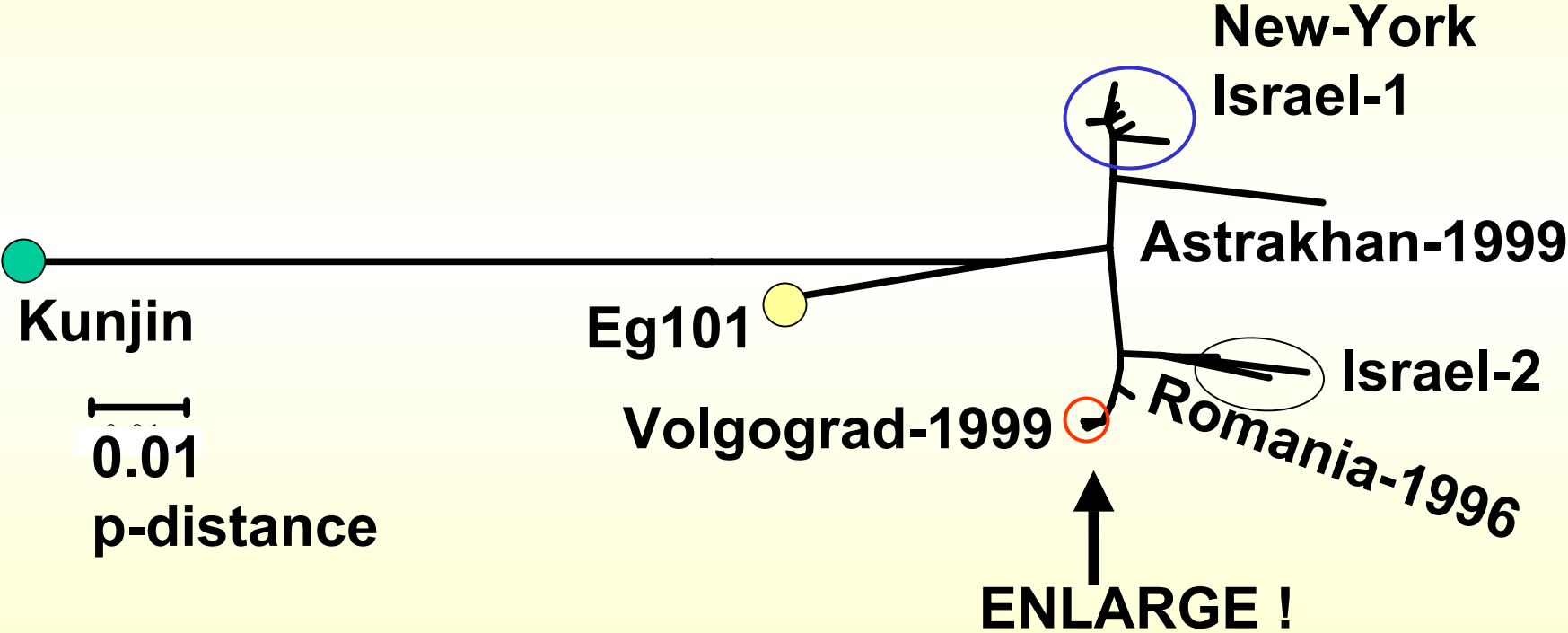
**Period of tissue sampling (death)**



**Total number of patients (July-October 1999) hospitalized with suspected WNF in Volgograd and Volzshski City and the time of brain tissue sampling from 14 patients**

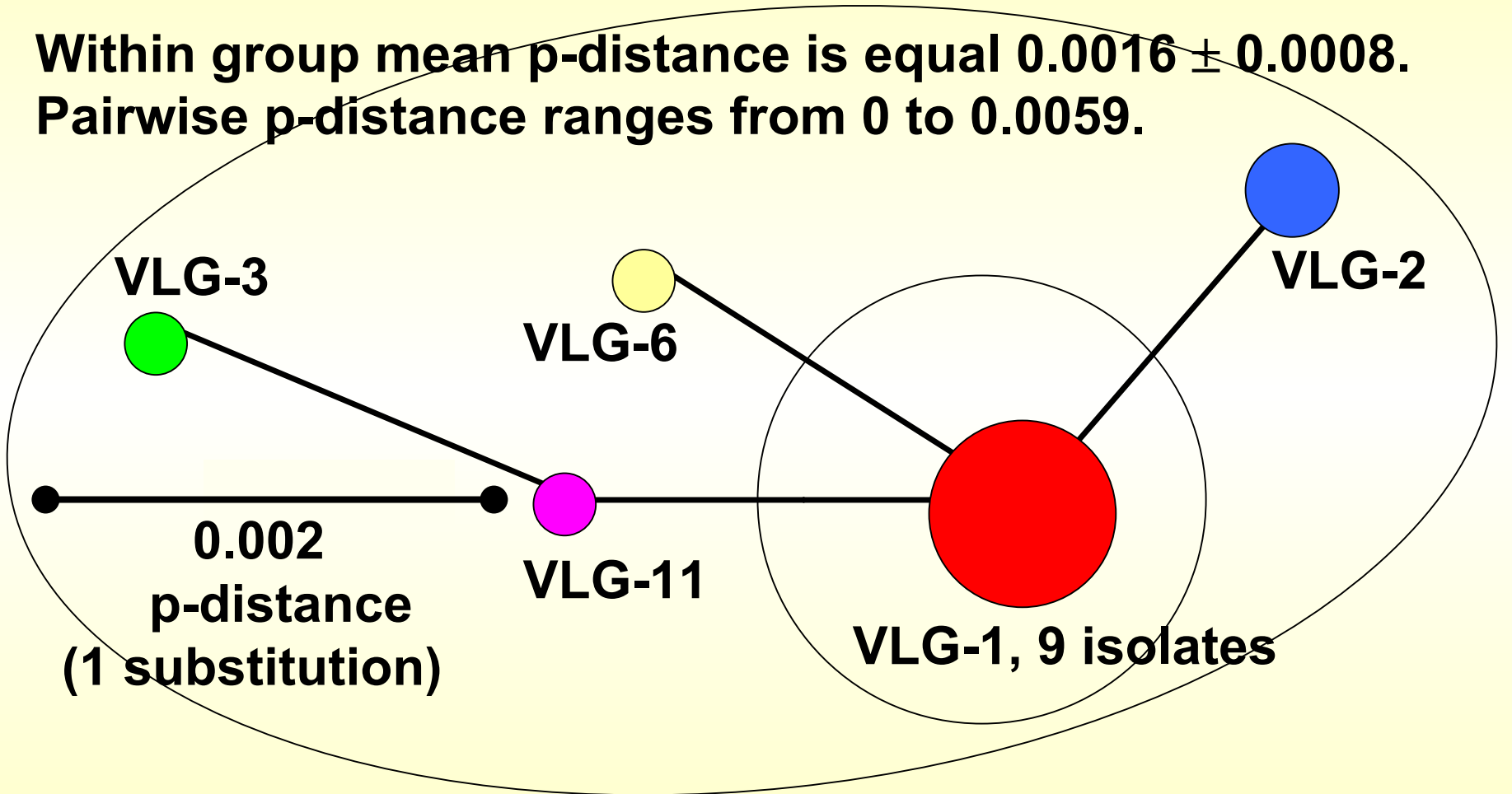


# Genetic tree based on envelope gene fragment, 512 nt, of West Nile virus isolates

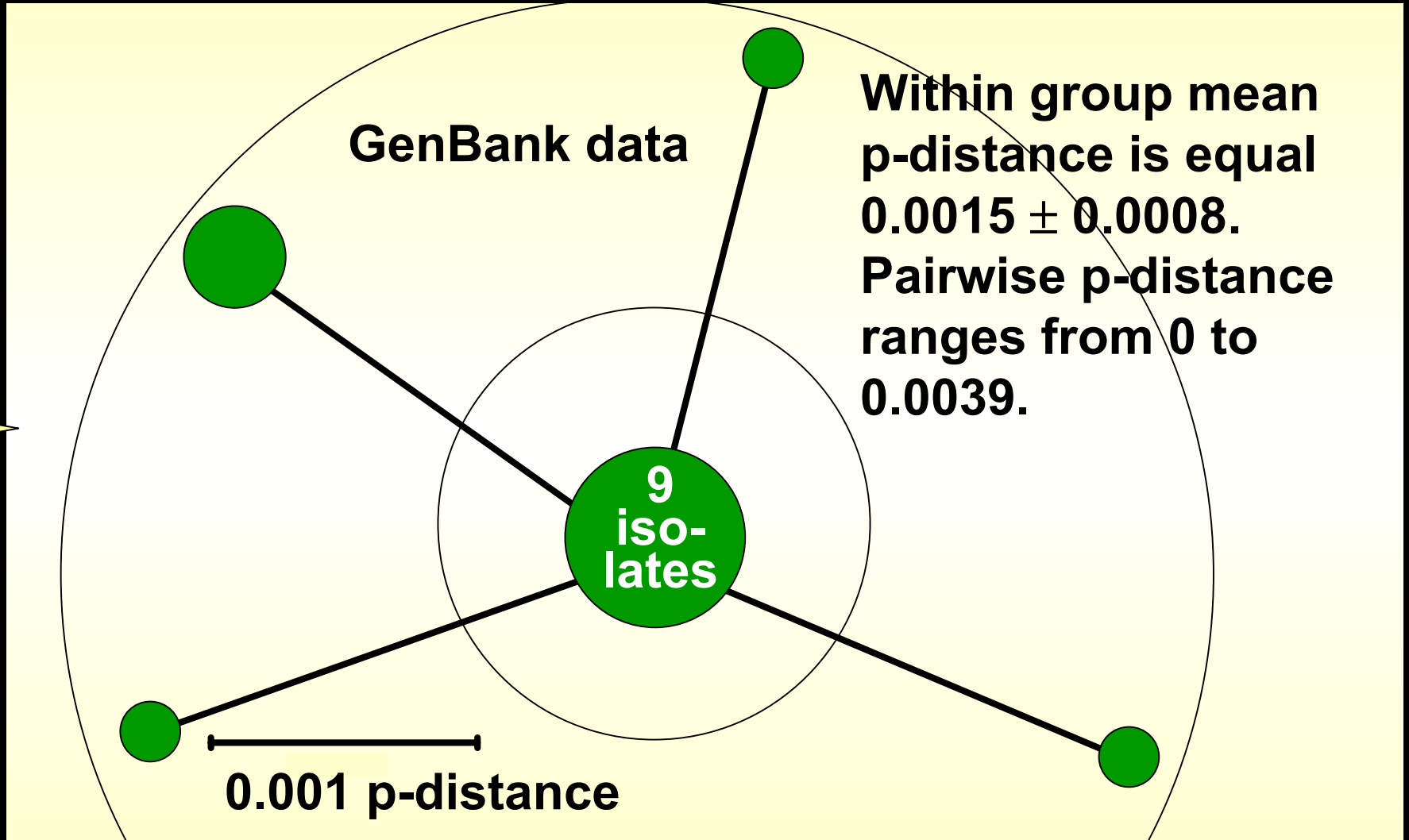


# Genetic tree based on envelope gene fragment, 512 nt, of WN virus isolates from Volgograd, 2.09.99-07.09.99

Within group mean p-distance is equal  $0.0016 \pm 0.0008$ .  
Pairwise p-distance ranges from 0 to 0.0059.

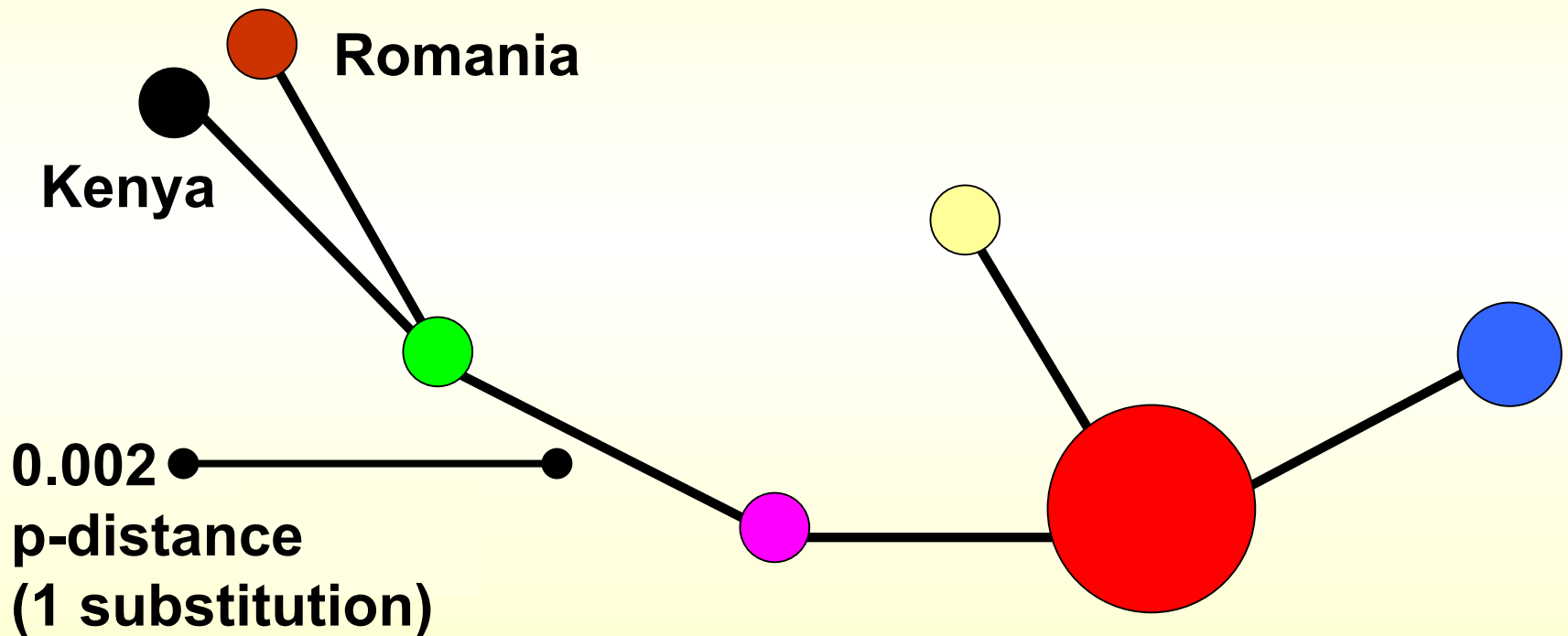


# Genetic tree based on envelope gene fragment, 512 nt, of 15 WN virus isolates from the USA, 1999-2000

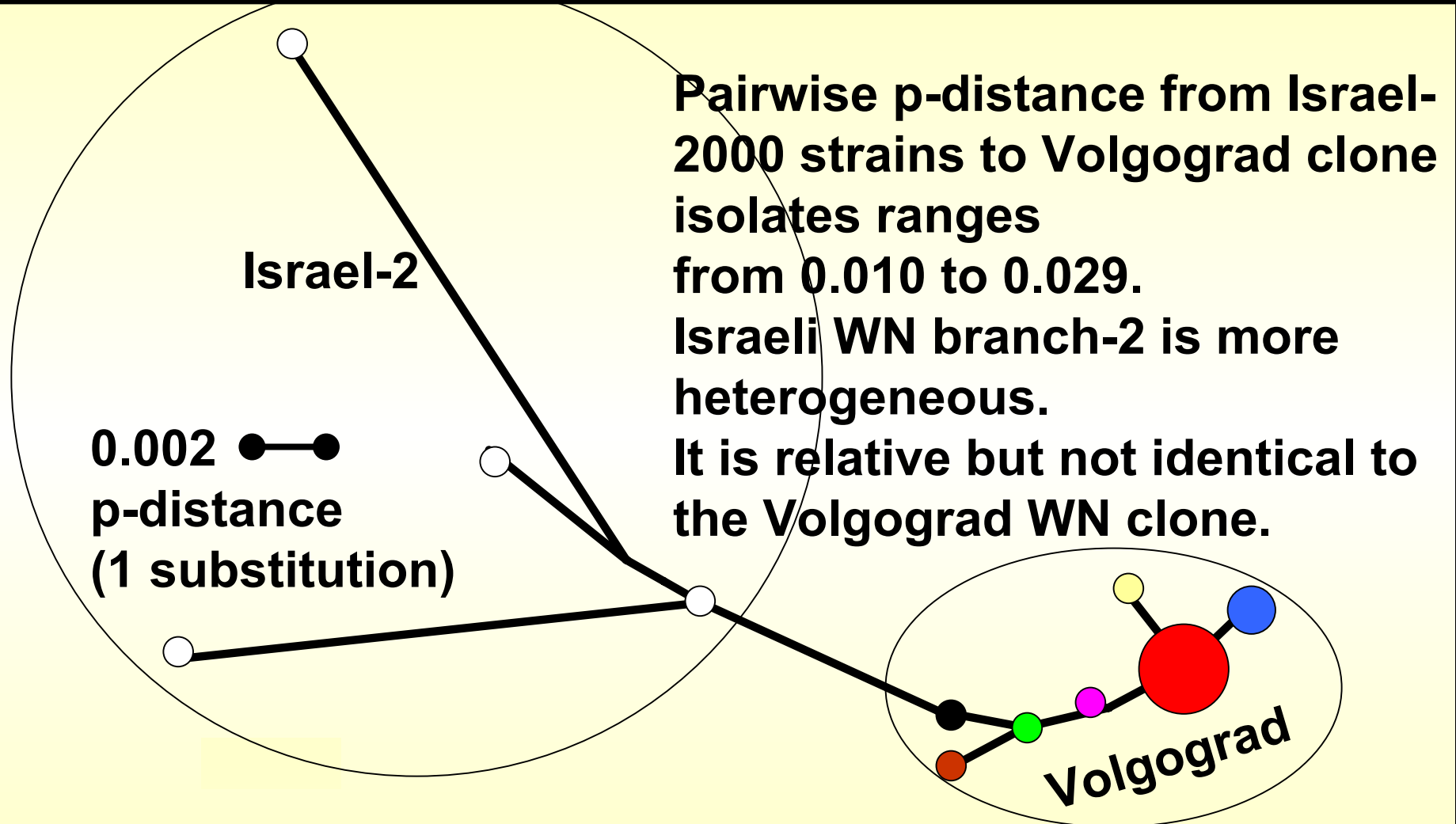


**Genetic tree based on envelope gene fragment, 512 nt,  
of WN virus isolates from Volgograd, 2.09.99-07.09.99,  
and mosquito strains from Romania and Kenya**

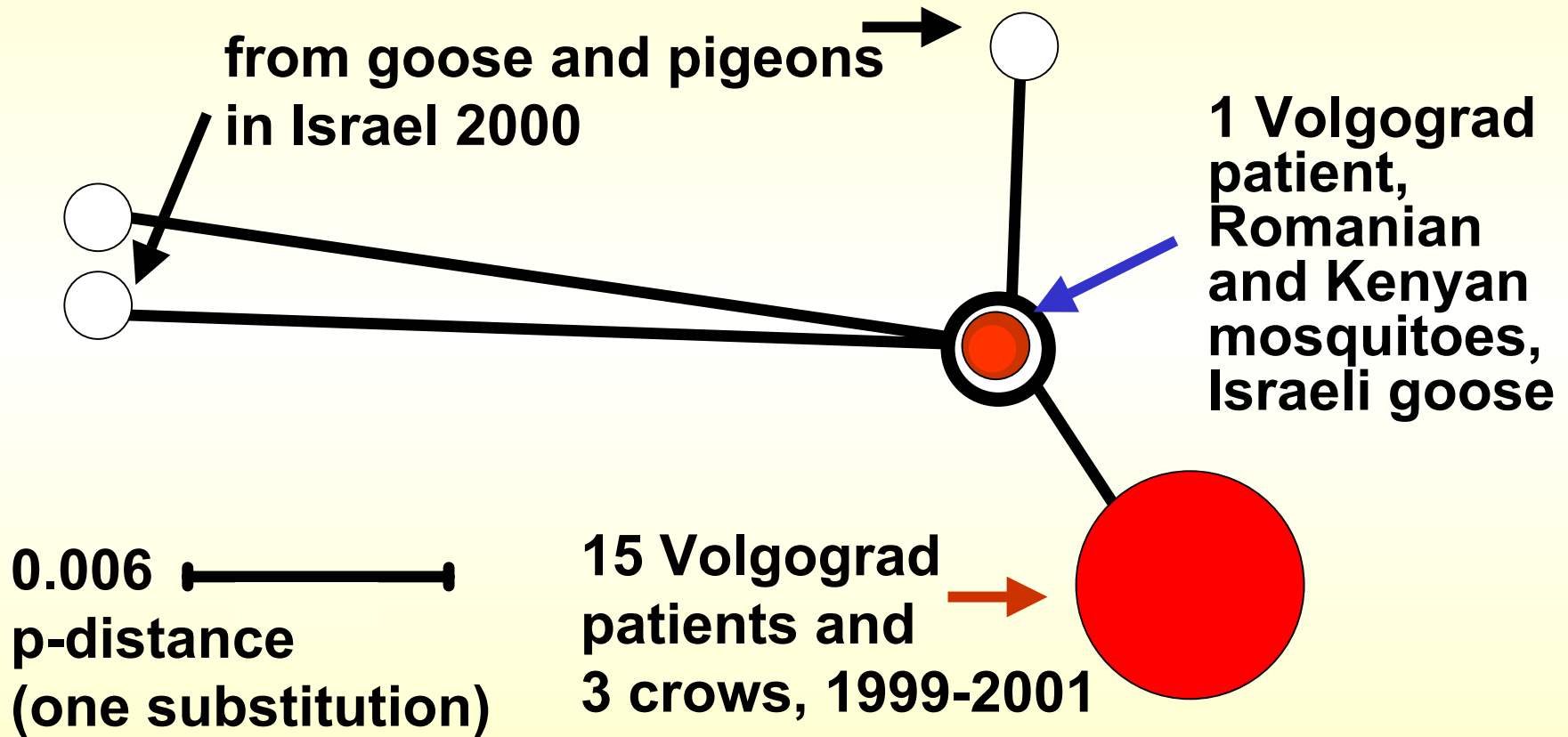
**Pairwise p-distance from Romania-1996 and Kenya-1998  
strains to Volgograd clone isolates  
ranges from 0.0020 to 0.0078.**



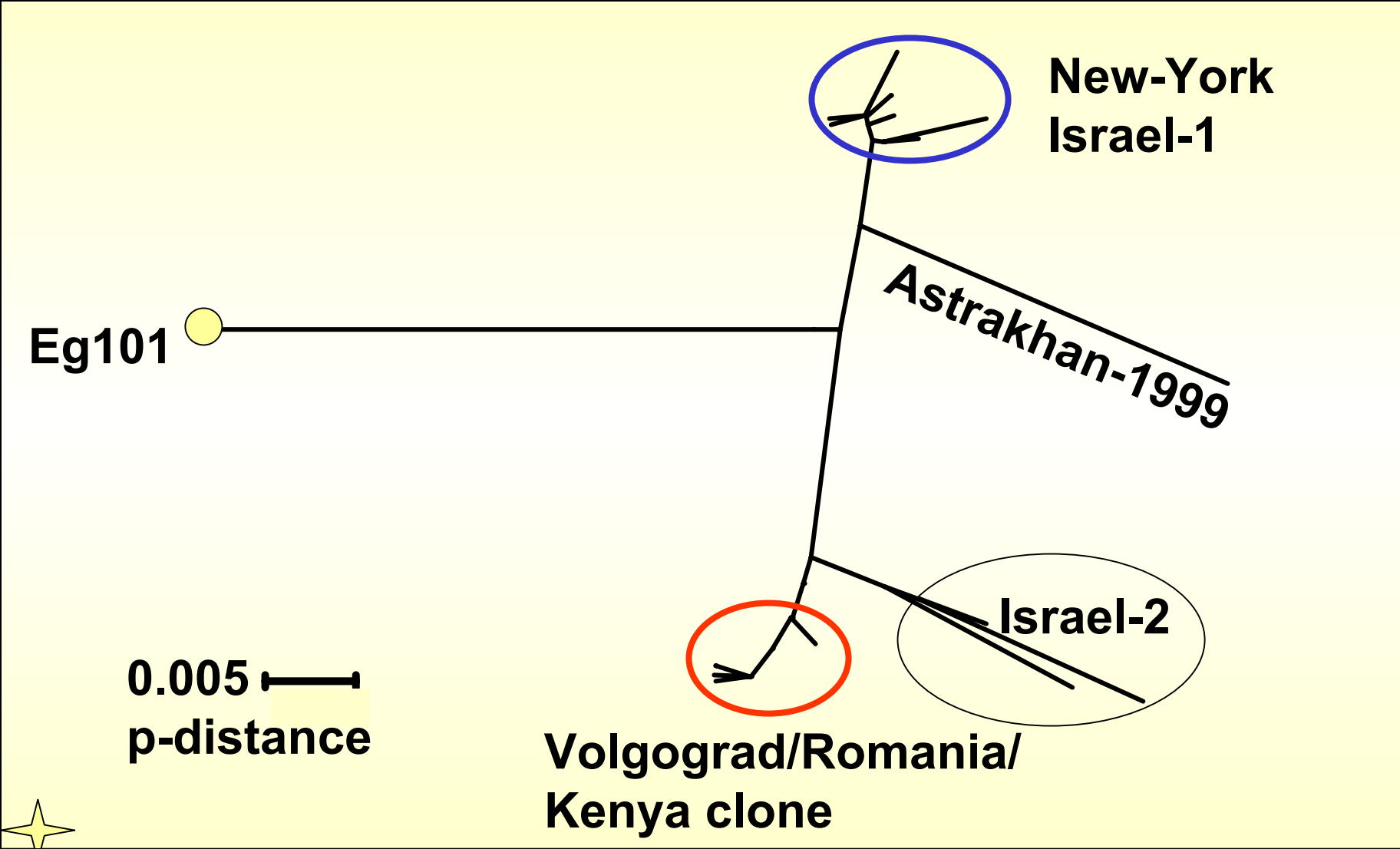
**Genetic tree based on envelope gene fragment, 512 nt,  
of WN virus isolates from Volgograd, 2.09.99-07.09.99,  
and strains from Romania, Kenya, and Israel**



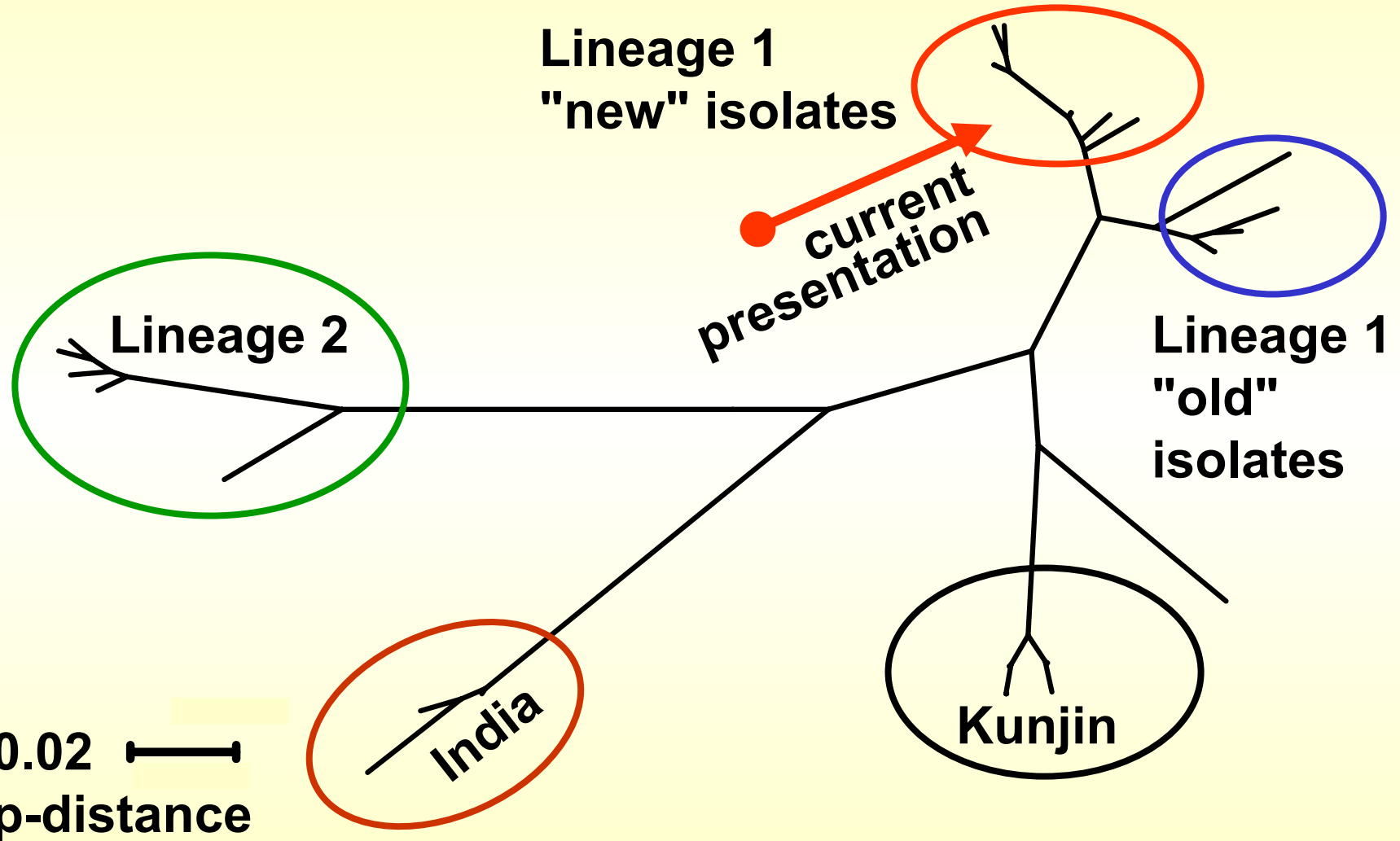
**Genetic tree based on 170 envelope amino acids,  
of WN virus isolates from Volgograd 1999, 2000,2001,  
Romaina, Kenya, Israel**



# Genetic tree based on envelope gene fragment, 512 nt, of West Nile virus isolates



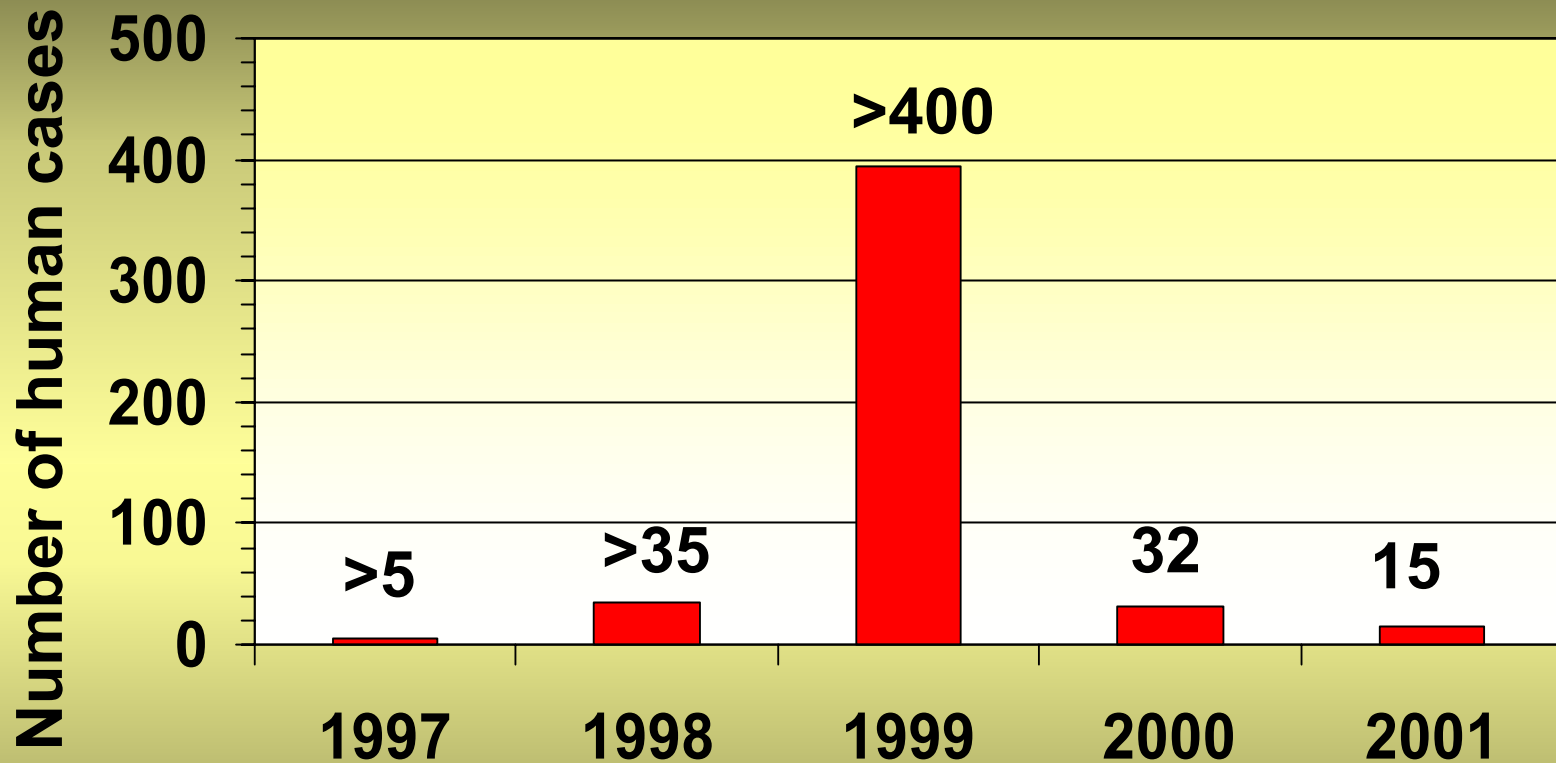
# Genetic tree based on envelope gene fragment, 254 nt, of West Nile virus isolates from all lineages





# Laboratory diagnosis of WNF in Volgograd region (1997-98 by IgG-ELISA, in 1999-2001 by IgM-ELISA or PCR)

West Nile fever appear to be endemic in Volgograd region because WN disease cases were diagnosed in 1997-2001



## **West Nile fever persists in Volgograd region because**

**WN-like antigen was found by ELISA in 1999-2000:**  
in 6 (3%) of 191 pools of *Culex pipiens* mosquitoes;  
in 6 (9%) of 67 pools of *Hyalomma scupence* ticks;  
in 2 (5%) of 43 brain tissue samples from *Corvus cornix* crows;  
in 9 (6%) of 159 brain tissue samples from *Mus musculus* mice;

**58 (8%) of 709 healthy adult donors had anti-WN IgG in 2000**



# Genetic tree based on envelope gene fragment, 512 nt, of WN virus isolates from Volgograd 1999, 2000, 2001

Volgograd WN clone appear to be endemic in Volgograd region because the same isolates were found in 1999-2001

