

Putative New West Nile Virus Lineage in *Uranotaenia unguiculata* Mosquitoes, Austria, 2013



Figure 1: *Uranotaenia unguiculata*
<http://seveccontrolmosquito.blogspot.co.at/2011/10/uranotaenia-unguiculata-nova-especie-de.html>

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Objective

In this study a putative new WNV lineage was identified in a pool of *Ur. unguiculata* mosquitoes collected in Austria in 2013. The complete polyprotein coding sequence was determined and phylogenetic analyses were performed.

Methods

Mosquitoes were trapped at Lake Neusiedl-Seewinkel in Burgenland State (Austria) and at Nesyt fishpond near Valtice (Czech Republic). Mosquito species were determined according to morphologic criteria with a total outcome of 94 female *Ur. unguiculata* mosquitoes. The mosquito pools were screened for the presence of flaviviral RNA, using universal flavivirus primers within the nonstructural protein 5 (NS5) encoding gene. One mosquito pool from Austria, captured in late August, proved positive for WNV. The complete polyprotein coding sequence including partial 5' and 3' noncoding ends of this novel strain was obtained. Phylogenetic neighbor-joining trees were generated with MEGA5 software, using ClustalW alignments, 1,000 replicates for bootstrap testing, and evolutionary distances computation with the p-distance model. Furthermore characterization of the encoding polyprotein was performed.

Table 1: Sequence identities between the newly identified WNV strain from Austria, WNV-Uu-LN-AT-2013, and other strains representing different WNV lineages*

Strain/lineage†	Nucleotide identity or amino acid identity, %, by strain/lineage‡										
	WNV-Uu-LN-AT-2013	1a	1b	1c/5	2	3	4	6 (Spain)§	7 (Koutango virus)	8¶	Usutu virus
WNV-Uu-LN-AT-2013		88.3	87.9	87.0	88.8	86.7	96.2	95.9	85.3	81.2	75.5
1a	76.2		97.6	93.4	94.0	90.4	88.6	91.7	89.2	92.4	76.3
1b	75.4	88.2		92.7	93.5	89.8	88.3	91.2	88.8	92.0	76.1
1c/5	76.3	80.5	79.7		92.1	88.8	87.4	89.1	87.7	91.2	76.1
2	77.0	79.8	79.6	79.1		90.9	89.2	92.6	89.3	92.0	76.0
3	75.9	78.3	77.3	77.3	78.7		87.0	91.4	86.6	89.2	75.5
4	82.8	76.6	76.0	76.2	76.9	76.5		95.0	85.5	81.0	74.7
6 (Spain)§	83.2	78.1	78.1	77.7	78.6	79.5	81.7		88.6	-	80.8
7 (Koutango virus)	75.1	77.7	77.4	77.0	77.8	76.3	75.6	78.0		86.8	75.3
8¶	72.7	78.4	78.0	77.3	78.4	77.7	72.6	-	77.4		76.3
Usutu virus	71.2	72.4	72.6	72.4	71.3	71.0	70.1	73.6	72.4	72.5	

*Alignments were performed by using ClustalW2 (<http://www.ebi.ac.uk/Tools/msa/clustalw2/>). WNV, West Nile virus; WNV-Uu-LN-AT-2013, West Nile virus strain *Uranotaenia unguiculata*-Lake Neusiedl-Austria-2013; -, comparison between lineages 6 and 8 was not possible because the available partial sequences do not cover the same nucleotide regions.

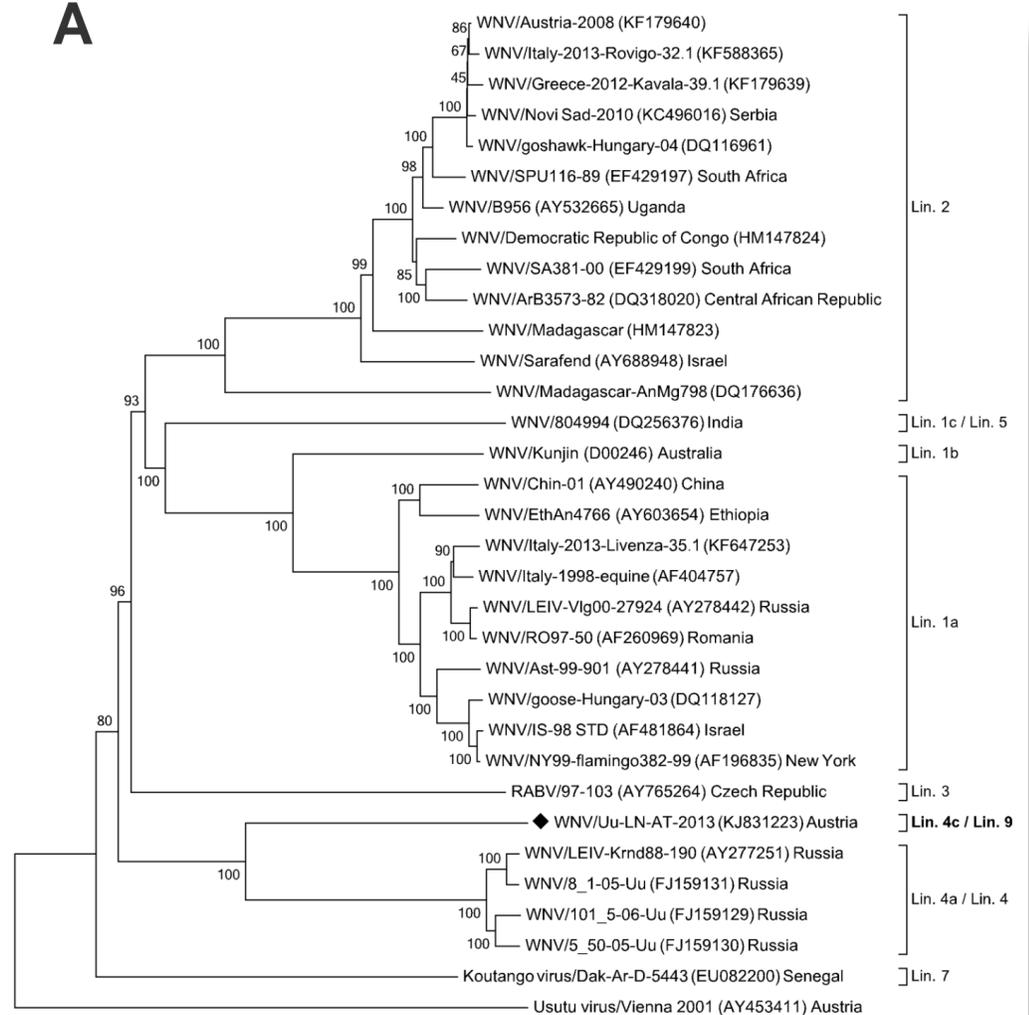
†GenBank accession nos. are as follows for the polyprotein genes/polyproteins: WNV-Uu-LN-AT-2013 (KJ831223), lineage 1a (AF404756/AAM81752), lineage 1b (D00246/BAA00176), lineage 1c (DQ256376/ABC40712), lineage 2 (DQ116961/AAZ91684), lineage 3 (AY765264/AAW81711), lineage 4 (FJ159129/ACH99530), lineage 6 (Spain) (GU047875/ADD69956), lineage 7 (Koutango virus) (EU082200/ABW76844), lineage 8 (KJ131502/AHV83443), Usutu virus (AY453411/AAS59402).

‡Amino acid sequences (above the diagonal) and nucleotide sequences (below the diagonal) are based on complete polyprotein genes, with the exception of lineage 6 and 8 strains, for which only partial sequences were available.

§Comparison was based only on partial NS5 gene sequences.

¶Comparison was based only on complete envelope protein gene sequences.

A



B

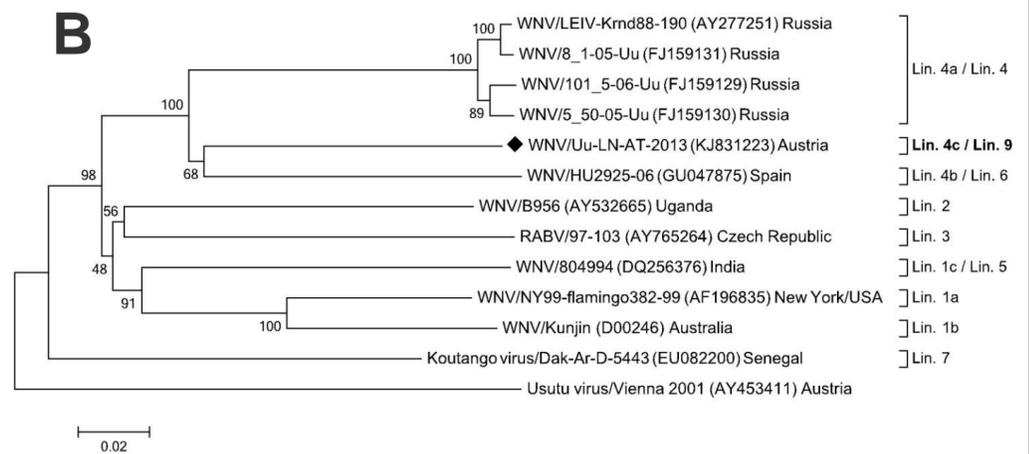


Figure 2: Phylogenetic positioning of WNV-Uu-LN-AT-2013, a West Nile virus (WNV) strain newly identified in Austria, within the species *West Nile virus*.

A) Phylogenetic position as determined on the basis of the full-length polyprotein-coding nucleotide sequences. B) Phylogenetic position as determined on the basis of 1,813-nt fragments of NS5, inferred by using the neighbor-joining method of MEGA5 with 1,000-fold bootstrap analysis, and rooted against the respective sequence of Usutu flavivirus. Numbers next to the branches indicate the percentage of replicates in the bootstrap analysis. Black diamond indicates the WNV sequence determined in this study. GenBank accession numbers are shown in parentheses with the virus names. Scale bars indicate nucleotide substitutions per site. Lin., lineage; RABV, Rabensburg virus.

Results

The complete polyprotein gene sequence of the newly detected WNV strain from Austria (WNV-Uu-LN-AT-2013) shares a maximum identity of $\approx 83\%$ with lineage 4 WNV strains isolated from *Ur. unguiculata* mosquitoes in Russia. At the amino acid level, the entire polyproteins of WNV-Uu-LN-AT-2013 and the lineage 4 strains from Russia share $\approx 96\%$ identity. Compared with the Russian lineage 4 strains, a 1,813-nt fragment of the NS5-coding sequence of a putative lineage 6 WNV, isolated from *Culex pipiens* mosquitoes in Spain, shares slightly higher nucleotide and amino acid identities with WNV-Uu-LN-AT-2013. *In silico* analysis, using NetNGlyc 1.0 software, yielded a single putative glycosylation site at N-154 of the envelope protein as well as three highly conserved glycosylation sites at positions N-130, N-175 and N-207 of NS1. Such alterations have been implicated in increased WNV pathogenicity and neuroinvasiveness [Whiteman MC *et al.*, Vaccine 2011 Dec 6;29(52):9702-10].

Conclusion

We suggest that the new WNV-Uu-LN-AT-2013 strain from Austria either constitutes a new lineage (lineage 9) or can be grouped into lineage 4 as sublineage 4c, with the strains from Russia and Spain as sublineages 4a and 4b, respectively.

