

Supplementary Materials

Table S1. Dataset of 198 WNV-L2 reference sequences used for the phylogenetic analysis of WNV lineage 2 strains.

GenBank	Country	Year	GenBank	Country	Year	GenBank	Country	Year	GenBank	Country	Year
MH924836	Germany	2018	MN481594	Greece	2012	MW751842	Serbia	2018	MN939563	Italy:Veneto	2018
KF179640	Austria	2008	MN481595	Greece	2010	MW751843	Serbia	2018	MN939564	Italy:Veneto	2016
KM659876	Austria	2014	MN481596	Greece	2011	MW751844	Serbia	2018	MW862073	Italy:Sardinia	2012
KP109691	Austria	2014	MN481597	Greece	2012	MW751845	Serbia	2018	MW862074	Italy:Sardinia	2013
KP109692	Austria	2014	MN652880	Greece	2018	MW751846	Serbia	2018	MW862075	Italy:EmiliaRomagna	2015
MF984337	Austria	2015	MT341470	Greece	2019	MH244510	Slovakia	2014	MW862076	Italy:Sardinia	2015
MF984338	Austria	2015	MT341471	Greece	2019	MH244511	Slovakia	2013	MW862077	Italy:Sardinia	2015
MF984339	Austria	2015	DQ116961	Hungary	2004	MH244512	Slovakia	2013	MW862078	Italy:Piedmont	2015
MF984340	Austria	2015	KC496015	Hungary	2010	MH244513	Slovakia	2013	MW862079	Italy:Piedmont	2015
MF984341	Austria	2015	KT359349	Hungary	2014	MW561633	Slovakia	2018	MW862080	Italy:Sardinia	2015
MF984342	Austria	2015	OK129329	Hungary	2016	OM037670	Spain	2017	MW862081	Italy:Sardinia	2015
MF984343	Austria	2015	OK129330	Hungary	2016	OM037671	Spain	2020	MW862082	Italy:Lombardy	2015
MF984344	Austria	2015	OK129331	Hungary	2015	OM037672	Spain	2020	MW862083	Italy:Sardinia	2015
MF984345	Austria	2015	OK129332	Hungary	2015	OM037673	Spain	2020	MW862084	Italy:Sardinia	2015
MF984346	Austria	2016	OK129333	Hungary	2017	MH939154	Romania	2014	MW862085	Italy:Sardinia	2015
MF984347	Austria	2016	OK129334	Hungary	2017	ON813230	Italy:EmiliaRomagna	2021	MW862086	Italy:Sardinia	2016
MF984348	Austria	2016	OK129335	Hungary	2017	ON813231	Italy:EmiliaRomagna	2021	MW862087	Italy:Sardinia	2017
MF984349	Austria	2016	OK129336	Hungary	2017	ON813232	Italy:Sardinia	2021	MW862088	Italy:Sardinia	2017
MF984350	Austria	2016	OK239658	Hungary	2018	ON813233	Italy:Piedmont	2022	MW862089	Italy:Lombardy	2017
MF984351	Austria	2016	OK239659	Hungary	2018	OP850024	Italy:EmiliaRomagna	2022	MW862090	Italy:Sardinia	2018
MF984352	Austria	2016	OK239660	Hungary	2018	OP850025	Italy:Piedmont	2022	MW862091	Italy:Sardinia	2018
KU206781	Bulgaria:Sofia	2015	OK239661	Hungary	2018	OP850026	Italy:Friuli	2022	MW862092	Italy:Sardinia	2018
MT341472	Bulgaria	2018	OK239662	Hungary	2018	OP850027	Italy:Lombardy	2021	MW862093	Italy:Sardinia	2018
KM203860	Cz Republic	2013	OK239663	Hungary	2018	OP850028	Italy:EmiliaRomagna	2021	MW862094	Italy:Sardinia	2019
KM203861	Cz Republic	2013	OK239664	Hungary	2018	OP850029	Italy:EmiliaRomagna	2022	MW862095	Italy:Sardinia	2019
KM203862	Cz Republic	2013	OK239665	Hungary	2018	OP734238	Italy:EmiliaRomagna	2022	MW862096	Italy:Lombardy	2018
KM203863	Cz Republic	2013	OK239666	Hungary	2018	OP734239	Italy:EmiliaRomagna	2022	MW862097	Italy:Sardinia	2018
MT863560	France	2018	OK239668	Hungary	2018	OP734240	Italy:Piedmont	2022	MW862098	Italy:Sardinia	2018
MT863561	France	2018	OK239669	Hungary	2017	OP734241	Italy:Piedmont	2022	MW862099	Italy:Sardinia	2018
MH986055	Germany	2018	OK239670	Hungary	2018	OP734242	Italy:EmiliaRomagna	2022	MW862100	Italy:Sardinia	2018
MH986056	Germany	2018	OK239671	Hungary	2018	OP734243	Italy:EmiliaRomagna	2022	MW862101	Italy:Sardinia	2019
MW142223	Germany	2020	OK239672	Hungary	2018	OP734244	Italy:Veneto	2022	MW862102	Italy:Veneto	2019
MW142224	Germany	2020	KP789953	Italy:Lombardy Pavia	2014	OQ204314	Italy:Tuscany	2022	MW862103	Italy:Piedmont	2019
MW142226	Germany	2020	KP789954	Italy:Lombardy Cremona	2014	OQ204315	Italy:Sigily	2022	MW862104	Italy:Piedmont	2019
MW142227	Germany	2020	KP789955	Italy:Veneto Verona	2014	KF588365	Italy:Veneto	2013	MW862105	Italy:Sardinia	2019
HQ537483	Greece	2010	KP789956	Italy:Veneto Verona	2014	KF647248	Italy:Veneto	2013	MW862106	Italy:Piedmont	2019
KF179639	Greece	2012	KP789957	Italy:Lomabrdy Cremona	2014	KF647249	Italy:Veneto	2013	MW862107	Italy:Piedmont	2019
KJ883343	Greece	2013	KP789958	Italy:Lombardy Pavia	2014	KF647250	Italy:Veneto	2013	MW862108	Italy:Piedmont	2020
KJ883344	Greece	2013	KP789959	Italy:Lombardy Pavia	2014	KF647251	Italy:Veneto	2013	ON032488	Italy:Piedmont	2020
KJ883348	Greece	2013	KP789960	Italy:Lombardy Mantova	2013	KF647252	Italy:Veneto	2013	ON032489	Italy:Piedmont	2020
KY594040	Greece	2010	MN939558	Italy:Veneto Rovigo	2016	KF823805	Italy:Veneto	2013	ON032490	Italy:Piedmont	2021
MN480792	Greece	2018	KC496016	Serbia	2010	KF823806	Italy:Lombardy	2013	ON032491	Italy:Lombardy	2021
MN480793	Greece	2018	MW751834	Serbia	2018	KT207792	Italy:Veneto	2014	ON032492	Italy:EmiliaRomagna	2021
MN480794	Greece	2018	MW751835	Serbia	2018	KU573080	Italy:EmiliaRomagna	2013	ON032493	Italy:EmiliaRomagna	2021
MN480795	Greece	2018	MW751836	Serbia	2015	KU573082	Italy:EmiliaRomagna	2013	ON032494	Italy:Sardinia	2021
MN481589	Greece	2018	MW751837	Serbia	2018	MN939557	Italy:Veneto	2018	ON032495	Italy:EmiliaRomagna	2021
MN481590	Greece	2018	MW751838	Serbia	2018	MN939559	Italy:Veneto	2016	ON032497	Italy:EmiliaRomagna	2021
MN481591	Greece	2018	MW751839	Serbia	2020	MN939560	Italy:Veneto	2016	PP625328	Italy:Sardinia	2022
MN481592	Greece	2012	MW751840	Serbia	2020	MN939561	Italy:Veneto	2018			
MN481593	Greece	2012	MW751841	Serbia	2018	MN939562	Italy:Veneto	2018			

Table S2. Primer pool 1 WNV-L2.

Primer Name	Sequence
WNV-L2_1_LEFT	GCCTGTGTGAGCTGACAAACTT
WNV-L2_1_RIGHT	TTCTTTTGTTTTGTGCTCCGGC
WNV-L2_3_LEFT	GTSYTRGCTGCTGGAAATGAYC
WNV-L2_3_RIGHT	CMACCCATGTAGCTCCAGAYAC
WNV-L2_5_LEFT	GTRGACAGRGGATGGGGAAAYG
WNV-L2_5_RIGHT	GRTTCCTCCAHGYGGTGCTT
WNV-L2_7_LEFT	GCDTTYAAATTCGCGYGGGACTC
WNV-L2_7_RIGHT	GTGTATRGCTTTCCTCCYACYGAG
WNV-L2_9_LEFT	CGGTGYGGAAGTGGAGTGTTYA
WNV-L2_9_RIGHT	TRYTRTTCATGCTCGGTTSR
WNV-L2_11_LEFT	CACHYTGTGGGGTGATGGAGTT
WNV-L2_11_RIGHT	CAGAAGGCCCAACTGAAAAGGA
WNV-L2_13_LEFT	CAGTCTTTCTGGTGGCTTCBTT
WNV-L2_13_RIGHT	YCCAGCKGCAAGTATCATBGGA

WNV-L2_15_LEFT	GGACRGCTGAYATYACYTGGA
WNV-L2_15_RIGHT	RCTCATGAGAGCRGCTCCCTTR
WNV-L2_17_LEFT	RGACTATCCCACYGGAACRTCA
WNV-L2_17_RIGHT	TGGCACATGACATCAACGATYT
WNV-L2_19_LEFT	ACMGAGCCTGGAACACTGGVTA
WNV-L2_19_RIGHT	CCTCCATAGCAATACTCATCACCA
WNV-L2_21_LEFT	CWGTCTGGCTCGCTTACAAAGT
WNV-L2_21_RIGHT	ARGCTATTGTCTGAAGGGCRTC
WNV-L2_23_LEFT	YCAGCTYGCCGTGTTTTGATY
WNV-L2_23_RIGHT	CYGCAGTCACAGTCACAGTCAG
WNV-L2_25_LEFT	ARRACTGTCAGAGAGGCYGGAA
WNV-L2_25_RIGHT	GCCTTTCCACTAACCACCGYAR
WNV-L2_27_LEFT	CACACTGCTCTGTGACATTGGA
WNV-L2_27_RIGHT	AGAATTGAGGAGAGGCTTCCCY
WNV-L2_29_LEFT	CTCCTTTCGGHCAACAACGRGT
WNV-L2_29_RIGHT	CYCCBAGCCACATGAACCADAT
WNV-L2_31_LEFT	CDAAGGTBCTTGARCTGCTDGR
WNV-L2_31_RIGHT	GGACCTTTGACATGGCATTBAGR
WNV-L2_33_LEFT	GCAGATGTGGCTGYTGCTTTAT
WNV-L2_33_RIGHT	YRTCTTCATACCTCCTCARDGA
WNV-L2_35_LEFT	GGAAGTTGAGTAGACGGTGCTG
WNV-L2_35_RIGHT	TCCCAGGTGTCAATATGCTGTT

Table S3. Primer pool 2 WNV-L2.

Primer Name	Sequence
WNV-L2_2_LEFT	ACAGCGATGAAGCATCTCTTGA
WNV-L2_2_RIGHT	GBCGDGTYTTDGTGCATCTYCC
WNV-L2_4_LEFT	ATNCTATTGCTCCTGGTRGCA
WNV-L2_4_RIGHT	TCCADCCAGTTGCTTTGGTKGW
WNV-L2_6_LEFT	CCTTCCTGGTYCACCGAGARTG
WNV-L2_6_RIGHT	KGAVGAAATGGGCACYTTRCAR
WNV-L2_8_LEFT	ACTCAGAGGAGCTCAACGACTC
WNV-L2_8_RIGHT	ATTYTTGCTAGGCCTTGCGHG
WNV-L2_10_LEFT	YGCDCCAGARCTAGCTAACAAYA
WNV-L2_10_RIGHT	CCCTGGYCTCCTGTTRTGRTGC
WNV-L2_12_LEFT	GAYGAAAAGACCCTCGTGCA
WNV-L2_12_RIGHT	CCATTTGRAAGAAAGCAGCTGCR
WNV-L2_14_LEFT	MRGTTGGAAGCYTCATCAARGARA
WNV-L2_14_RIGHT	TGAAAATTTCCATCRTCATCCARCC
WNV-L2_16_LEFT	ATHATGACTCGAGGTCTGCTYG
WNV-L2_16_RIGHT	MRCCATTDGGCATGATGACKCC
WNV-L2_18_LEFT	TKAGAGGACTTCCCATYCGGTA
WNV-L2_18_RIGHT	TTBCCCATTTTCACACTTGGAACR
WNV-L2_20_LEFT	TGATGGAAGAGTCATCCTGGGV
WNV-L2_20_RIGHT	GTGTTGGTTGAGGTCCATCAA
WNV-L2_22_LEFT	TTKGACACGATGTATGTGGTKG
WNV-L2_22_RIGHT	CAYTCTTGGTCTTGTCAGCCA
WNV-L2_24_LEFT	YTTTGTVGACGTTGGTGTGTCA
WNV-L2_24_RIGHT	TYGTTGCRTTCCACACTGARCT
WNV-L2_26_LEFT	RHGCCAGGAGAGAGGGAAAYRT

WNV-L2_26_RIGHT	CCARTCTTCCACCATCTCCARR
WNV-L2_28_LEFT	AAGAAAACVTGGAAGGGACCYC
WNV-L2_28_RIGHT	TBGTGGTCTCATTGAGGACGTR
WNV-L2_30_LEFT	ACYTGCATCTACAACATGATGGG
WNV-L2_30_RIGHT	GGBCTCATCACTTTCACGACYT
WNV-L2_32_LEFT	GGHGATGACTGYGTGGTDAA
WNV-L2_32_RIGHT	GRACCCAGTTVACAGGCACA
WNV-L2_34_LEFT	GCGCHACTTGGGCTGAAAAYAT
WNV-L2_34_RIGHT	MYCTTCCGAGACGGTTCTGA