

Supplementary online material

Supplementary online table 1: List of samples, assigned haplotype, origin, type of sample (field collection or laboratory stock) and GenBank accession number. Results of *Wolbachia* analysis: U = uninfected, A = only strain wNvitA , AB = doubly infected for strains wNvitA and wNvitB.

Sample ID	haplotype	<i>Wolbachia</i> analysis	state and country	location	sample type	GenBank Accession No.
CH-2	8	AB	Switzerland	Langenthal	field	EU935412
FIG-1-FRA	9	AB	France	INRA-Gotheron Valence	laboratory	EU935344
Bay-1-GER	2	AB	Germany	Bayreuth	field	EU935408
Bay-3-GER	2		Germany	Bayreuth	field	EU935409
S-04-GER	8		Germany	Schlüchtern	field	EU935341
S-73-GER	2		Germany	Schlüchtern	field	EU935370
S-89-GER	8		Germany	Schlüchtern	field	EU935343
S-146-GER	8		Germany	Schlüchtern	field	EU935342
S-159-GER	7		Germany	Schlüchtern	field	EU935338
HH-1-GER	9		Germany	Hamburg	field	EU935339
HH-2-GER	3	AB	Germany	Hamburg	field	EU935346
HH-3-GER	9		Germany	Hamburg	field	EU935331
HH-4-GER	7	AB	Germany	Hamburg	laboratory	EU935347
HH-6-GER	7		Germany	Haseldorf	field	EU935332
HH-8-GER	8		Germany	Bad Arolsen	field	EU935336
HH-9-GER	15	AB	Germany	Eberdingen	field	EU935340
HH-10-GER	8		Germany	Bad Mergentheim	field	EU935361
HH-11-GER	9		Germany	Elmshorn	field	EU935345
ITA-1	22	AB	Italy	Toscany	field	EU935406
ITA-4	23	AB	Italy	Piemont	field	EU935407
AWD-1-NL	1	A*	Netherlands	Amsterdam	laboratory	EU935326
BU-2000-NL	1		Netherlands	Bussum	laboratory	EU935411
SPA-1-NL	1		Netherlands	Bussum	laboratory	EU935327

EP-B5-NL	6	U*	Netherlands	Elspeet	laboratory	EU935334
EL-13-NL	7	AB	Netherlands	Elspeet	laboratory	EU935335
HV-4-NL	10		Netherlands	Hoge Veluwe	field	EU935348
HV-527-NL	16	AB	Netherlands	Hoge Veluwe	field	EU935362
HV-736-NL	24	AB	Netherlands	Hoge Veluwe	field	EU935410
HV-815-NL	7		Netherlands	Hoge Veluwe	field	EU935337
HV-836-NL	2		Netherlands	Hoge Veluwe	field	EU935363
RU-BT-RUS	17	AB	Russia	Moscow	laboratory	EU935369
RU-277-RUS	18	AB	Russia	Moscow	laboratory	EU935371
US-7-CN	2		Ontario- CN		laboratory	EU935388
US-23-CN	19		Ontario- CN		laboratory	EU935391
US-41-CN	2		Ontario- CN		laboratory	EU935400
US-17-ID	2		Idaho USA		laboratory	EU935376
US-28-ID	2		Idaho USA		laboratory	EU935384
US-38-ID	2		Idaho USA		laboratory	EU935398
US-43-ID	2		Idaho USA		laboratory	EU935402
US-1-IN	5		Indiana USA		laboratory	EU935353
US-20-IN	2		Indiana USA		laboratory	EU935379
US-3-MI	2		Michigan USA		laboratory	EU935385
US-8-MI	2		Michigan USA		laboratory	EU935389
US-9-MI	5	A*	Michigan USA		laboratory	EU935355
US-21-MT	2		Montana USA		laboratory	EU935380
US-4-MT	2		Montana USA		laboratory	EU935386
US-33-NV	2		Nevada USA		laboratory	EU935396
US-15-NY	2		New York USA		laboratory	EU935374
US-29-NY	2	U*	New York USA		laboratory	EU935392
US-34-NY	2		New York USA		laboratory	EU935397
US-45-NY	2		New York USA		laboratory	EU935413

US-47-NY	2		New York USA		laboratory	EU935403
US-49-NY	2		New York USA		laboratory	EU935404
BR-001-NY	2		New York USA	Brewerton	field	EU935328
BR-38-NY	11	AB	New York USA	Brewerton	field	EU935349
BR-44-NY	11		New York USA	Brewerton	field	EU935360
BR-153-NY	5		New York USA	Brewerton	field	EU935333
BR-219-NY	2		New York USA	Brewerton	field	EU935329
BR-223-NY	2		New York USA	Brewerton	field	EU935330
BR-415-NY	2		New York USA	Brewerton	field	EU935359
BR-442-NY	2		New York USA	Brewerton	field	EU935350
IT-1-NY	12		New York USA	Ithaca	field	EU935351
IT-20-NY	2		New York USA	Ithaca	field	EU935414
IT-21-NY	2		New York USA	Ithaca	field	EU935364
IT-39-NY	2		New York USA	Ithaca	field	EU935365
M-04-NY	2		New York USA	Mumford	field	EU935366
US-37 NY	13	U*	New York USA		laboratory	EU935357
US-26-OH	2		Ohio USA		laboratory	EU935383
US-5-OR	14		Oregon USA		laboratory	EU935358
US-6-PE	2		Pennsylvania USA		laboratory	EU935387
US-18-PE	2		Pennsylvania USA		laboratory	EU935377

US-31-PE	2		Pennsylvania USA		laboratory	EU935394
US-32-PE	2		Pennsylvania USA		laboratory	EU935395
US-36-PE	5		Pennsylvania USA		laboratory	EU935356
US-39-PE	2		Pennsylvania USA		laboratory	EU935399
US-2-SD	2		South Dakota USA		laboratory	EU935390
Mon-04-UT	5		Utah USA	Huntsville	field	EU935352
Mon-05-UT	2		Utah USA	Huntsville	field	EU935367
Mon-06-UT	2		Utah USA	Huntsville	field	EU935368
SL-08-UT	2		Utah USA	Strawberry Lake	field	EU935372
US-14-UT	2		Utah USA		laboratory	EU935373
US-16-UT	2		Utah USA		laboratory	EU935375
US-19-UT	2		Utah USA		laboratory	EU935378
US-25-UT	2		Utah USA		laboratory	EU935382
US-30-UT	2		Utah USA		laboratory	EU935393
US-42-UT	20	A*	Utah USA		laboratory	EU935401
US-52-UT	21		Utah USA		laboratory	EU935405
US-10-VI	5		Virginia USA		laboratory	EU935354
US-24-WY	2		Wyoming USA		laboratory	EU935381

Supplementary online table 2: List of the strains used for sequencing nuclear markers.

		Strain	Locality
North	1	NV_CD12	Canada
American strains	2	NV_IN226	Indiana (USA)
	3	NV_Mn206B1	Minnesota (USA)
	4	NV_NVPA213N	Pennsylvania (USA)
	5	NV_OH204AK	Ohio (USA)
	6	NV_OR27	Oregon (USA)
	7	NV_R511	New York (USA)
	8	NV_RO64	Rochester, NY (USA)
	9	NV_XIDB409E	Idaho (USA)
	10	NV_XUTC402C	Utah (USA)
European strains	1	NV_AsyncX	Leiden (Netherlands)
	2	NV_ELB	Elspeet (Netherlands)
	3	NV_FIG	Valence, France
	4	NV_HV267_1	Hoge Veluwe, (Netherlands)
	5	NV_ITA002	Piedmont, Italy
	6	NV_NVEUS7	Sweden
	7	NV_Russia277	Zvenigorod (Russia)
	8	NV_RussiaBait	Moscow (Russia)

Supplementary online table 3: Microsatellite primer pairs used in this study. For each primer the chromosome, sequence, number of observed alleles, annealing temperatures and GENBANK accession numbers are given.

Locus	Chromosome	Primer sequences	Allele#	Ann. Temp.	GENBANK accession No.
Nv 22	I	5' GAC TGC GTA CCA CTC CAA AAA TA 3' 5' AAG ACC AGC TAG GGA AGA GGA TA 3'	14	58 °C	AY262041
Nv 23	II	5' ATA CTC AAG CAA GCC ACA GCA TA 3' 5' GCG TAC CAA TCC ACA GAA AAT AG 3'	17	58 °C	AY262044
Nv 41	IV	5' GTC AGA CGT GGG CTT TGT C 3' 5' TTA TGC GCC ACA CAC ACC 3'	15	52 °C	EU155141
Nv 46	V	5' TTA CGT CAA GGT ATA GCT GC 3' 5' GAA TAA GTG GCT GAA AGT TTC C 3'	12	58 °C	EU155142
Nv-44	I	5' CCA CTC GAT CGA TTA TTC CT 3' 5' GTG GCC AAT AGT TCA CAT CAA 3'	6	58 °C	FJ156233
Nv 300 ¹	II	5' ACA TTC CGC AGA GCG ATT AT 3' 5' CGC GAC CGA TGA TTT ACT C 3'	6	57 °C	FJ156211
Nv 303	III	5' GAC AAT AGC CGC TAC GGA AA 3' 5' CGT CGT TCT GCT GCT TCT C 3'	6	57 °C	FJ156214
Nv 313	V	5' GAA GCT GCG GGT TAA GTG TG 3' 5' CGC TAC TTT ATG CCA GTT ACG G 3'	16	57 °C	FJ156221
Nv 316	III	5' ACC AGA GAG GGG GAT TTC G 3' 5' CGC AGG ACA ACA TCA AAT A 3'	11	57 °C	FJ156228

¹ Nv 300 is a trinucleotide repeat marker, all others are dinucleotide repeat markers

Supplementary online table 4: List of the nuclear markers used in this study with their NCBI gene ID, location, primer sequence and the length of the amplified products.

Gene description	NCBI gene ID	Chromosome	Primer name	Sequence(5'-3')/Reference	Product length
similar to ENSANGP00000031746	LOC100119303	1	31746F 31746R	ATGGCATTAACTTCTTCGAATC GTTATCTTTACATCATCGATTGG	701
similar to Juvenile hormone-inducible protein putative	LOC100121269	1	JHIPF JHIPR	TCATCGGACTCTGGTGGTCG CGGTCATCACATCTCCATTTAC	828
similar to putative accessory gland protein	LOC100119650	2	ACPF ACPR	CCAGGTGAAAATCCTGTTGG TATGCCAGGGAAAACCATC	761
similar to ENSANGP00000012279	LOC100122234	2	12279F 12279R	AGGCTGCTTTCTTGGTGC TGATGAACTCGTTCTCCTGG	756
similar to 60S ribosomal protein L7/L12	LOC100118391	3	SRbpF SRbpR	TGACAATTCCAGTACCAGAAGGA AGTACTCAACATACAAGTTAATCTAAGG	547
Similar to arp2/3 complex	LOC100118949	4	WD1201- 20DF1 WD1201- 20DR1	Raychoudhury <i>et al.</i> (2009)	700
Similar to Lipase	LOC100118843	4	84J03SPR 84J03SPF	Raychoudhury <i>et al.</i> (2009)	402

Mitochondrial hydrogen-transporting ATP synthase,coupling factor F	LOC100114299	4	ATPFa ATPFb	Raychoudhury <i>et al.</i> (2009)	764
Similar to Casein Kinase I, alpha I	LOC100114338	5	CK1F1 CK1R1	Raychoudhury <i>et al.</i> (2009)	904
Similar to fumarylacetoacetate hydrolase	LOC100117860	5	FAA-F FAA-R	Raychoudhury <i>et al.</i> (2009)	721
similar to p27BBP/eIF6-like	LOC100115084	5	BBPF BBPR	GGCACATGATAAGAAAGATCCTTGCC GCGTATCTGCTAAAATTTCTTCCG	625

Supplementary online table 5: Comparison of the mitochondrial DNA results with Opijnen *et al.* (2005).

	this study	Opijnen <i>et al.</i> 2005
sequence	399bp COX I	349bp COX I
nucleotide diversity π	NV-EU: 0.0236 NV-NA: 0.0031 NV-World: 0.0149	NV: 0.0046 NG: 0.0127 NL: 0.0114
sample size	32 from EU 57 from NA	NV 2 from EU 18 from NA
Tajima's D	NV-EU: 0.53 n.s. NV-NA: -0.88 n.s. NV-World: -0.66 n.s.	NV: -1.81 p < 0.05 NG: 0.22 n.s. NL: 0.01 n.s.

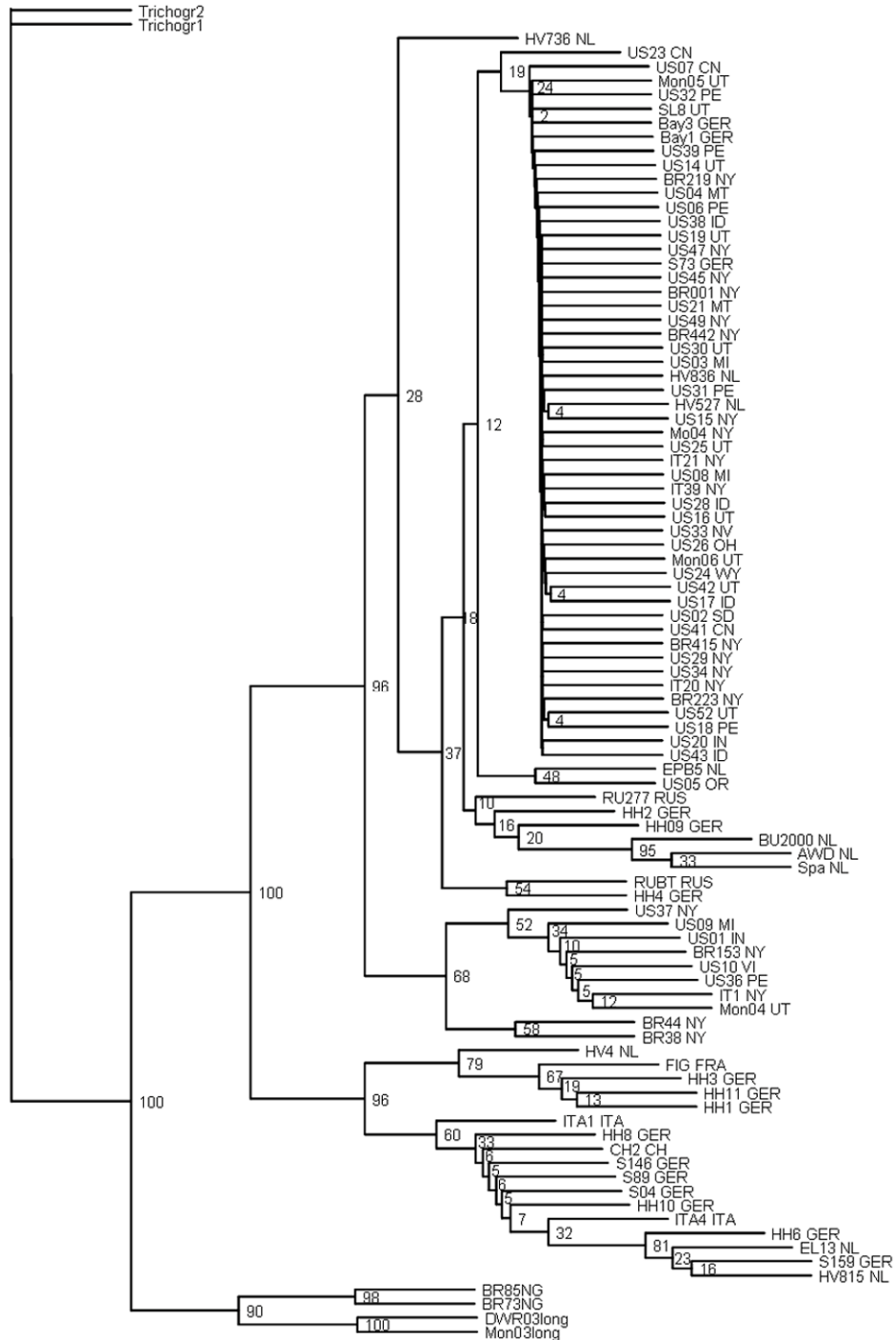
NV-EU = *N. vitripennis* Europe, NV-NA = *N. vitripennis* North America, NV-World = all *N. vitripennis* samples pooled, NL = *N. longicornis*, NG = *N. giraulti*.

Supplementary online table 6: Average number of nucleotide substitutions per site between populations using Jukes Cantor correction ($D_{XY} \pm SD$, Nei, 1987) based on mt-DNA. The numbers in brackets following the sample abbreviation indicate the sample size.

	NV-EU(32)	NL(2)	NG(2)
NL(2)	0.136±0.021		
NG(2)	0.147±0.022	0.099±0.050	
NV-NA(57)	0.023±0.003	0.137±0.016	0.148±0.017
NV-World(89)		0.136±0.013	0.148±0.014

NV-EU = *N. vitripennis* Europe, NV-NA = *N. vitripennis* North America, NV-World = all *N. vitripennis* samples pooled, NL = *N. longicornis*, NG = *N. giraulti*.

Supplementary online figure 1: Neighbour joining tree based on the 399 bp mitochondrial *COI* sequence. Genetic distances were calculated using the F84 method in DNADIST (PHYLIP). Branch lengths and internal values indicate bootstrap values out of 100 bootstraps. The tree is rooted in an outgroup formed by Trichogr1 and Trichogr2 (*Trichogramma*). Sequences of the two sister species Mon03long, DWR03long (*N. longicornis*), BR85NG, BR73NG (*N. giraulti*) were added for comparison.



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Supplementary online figure 2: Unrooted Neighbour joining tree based on Nei's genetic distance D_S (Nei, 1987) between subpopulations calculated on the basis of nine polymorphic microsatellites. Branch lengths indicate genetic distance, numbers at branches the bootstrap value after 1000 permutations. Numbers in brackets indicate the number of individuals combined in the subpopulation. Germany = GER; Netherlands = NL; Switzerland, Italy and France = CH-IT-FR; Canada = CN; Idaho = ID; New York = NY; Utah = UT; Montana, Oregon, Wyoming, Nevada and South Dakota = MT-OR-WY-NV-SD; Pennsylvania and Virginia = PE-VI; Indiana, Michigan, Ohio = IN-MI-OH, Russia = RU. A tree based on Goldstein's $\delta\mu^2$ (Goldstein and Pollock, 1994) showed a similar topology (not shown).

