

**Table S1.** Sample sites information and number of individuals in the *M. longidorsalis*

Location	Site ID	Water system	N	Location
Jecheon stream	JC	Namhangang River	20	37°09'51"N 128°06'24"E
Hongjeongcheon stream	PC	Namhangang River	20	37°36'58"N 128°22'27"E
Gapyeongcheon stream	GP	Bukhangang River	20	37°49'48"N 127°31'03"E
Hongcheon River	HC	Bukhangang River	20	37°40'48"N 127°51'30"E
Jojongicheon stream	CP	Bukhangang River	20	37°43'56"N 127°25'15"E
Yeongpyeongcheon stream	PHC	Imjingang River	20	38°03'35"N 127°23'14"E

N: Number of samples.

**Table S2.** Summary microsatellite screening and raw data of long and short read sequencing in *M. longidorsalis*.

Raw data	Total reads	Total reads length (bp)
Long reads raw data	5,468,650	33,151,752,272
Short reads raw data	725,452,696	109,120,602,840
Microsatellite search		
Contig		6,220
Total size of examined sequences (bp)		1,116,379,304
Total number of identified SSRs		886,829
Number of SSR containing sequences		5,004
Number of sequences containing more than 1 SSR		4,898
Number of SSRs present in compound formation		414,225

**Table S3.** Microsatellite 19 loci information developed from *M. longidorsalis*

Locus	Primer sequence (5'→ 3')	Motif repeat	Product size (bp)	Dye	N	N <sub>A</sub>	H <sub>O</sub>	H <sub>E</sub>	PIC	GenBank Accession no.
Milo2	F: TGTAACGACGGCCAGTCCGAGAGCTGGATTCGA CAA R: TCCTGCTTCCAACCAAAGGT	(AC)17	158	FAM	27	15	0.741	0.899	0.873	OR722786
Milo9	F: TGTAACGACGGCCAGTTCGACACTGGGTAAAA ACTGT R: GTGTTTTTCACCCAGTGTGA	(ATA)9	188	FAM	26	14	0.654	0.878	0.846	OR722787
Milo11	F: TGTAACGACGGCCAGTGGGACTTCAATGGGAG GCAA R: CAGCCCTGACTCATGAACGA	(AC)10	202	FAM	27	13	0.519	0.912	0.886	OR722788
Milo13	F: TGTAACGACGGCCAGTATCTCCGCACACATCGA CTC R: TTAGCGTCTCCTGGGAGTGA	(AC)12	208	FAM	27	7	0.815	0.776	0.726	OR722789





Milo56	F: TGTAACGACGGCCAGTAGCAAGACCCTAGCAAC CAC  R: TGAGTGGTTGCTACGCAGTT	(ATTG)6	309	VIC	26	8	0.654	0.662	0.624	OR722800
Milo61	F: TGTAACGACGGCCAGTAGCAGAGCACGTTACAC ACA  R: CCCTAACCTCCACAGCGTT	(AC)10	323	VIC	26	3	0.154	0.277	0.253	OR722801
Milo63	F: TGTAACGACGGCCAGTAACATGGCGGACAACA AAGC  R: GAGGAGCTGGTTGAGAGTCG	(AGC)9	329	VIC	26	6	0.308	0.315	0.300	OR722802
Milo64	F: TGTAACGACGGCCAGTAATGATGCTCCCAGGCA ACA  R: CATTCGGCTCCTCCTCCATC	(TGG)9	331	VIC	26	7	0.769	0.692	0.634	OR722803
Milo80	F: TGTAACGACGGCCAGTGGATAGTCTCACGCCTT CGG  R: TTGTGCAGTCCATCAACCCA	(AC)13	365	VIC	26	11	0.577	0.851	0.813	OR722804

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N: Number of samples,  $N_A$ : Number of alleles,  $H_O$ : Observed heterozygosity,  $H_E$ : Expected heterozygosity, PIC: Polymorphic information content.