

Supplementary Information

Table S1. Primer sets for selected 76 microsatellite loci (UP: unambiguous and polymorphic locus, SL: selected locus for marker optimization, GAn: Genbank accession number).

No.	Locus	Primer Sequence	Repeated Motif	Sequence Size	Remarks
1	10203F	GCGTTGAGCACTGTACCTGA	(TG) ₁₃	227	UP, SL GAn: KT591166
	10203R	TTCAAAGTGTGGTACCGTAAAA			
2	33136F	GGCAGTAGTGGGAAATTGTCA	(CAT) ₈	225	UP, SL GAn: KT591167
	33136R	GCCTGCCACATAAAAGTCCT			
3	63105F	CTCACGAGCCTTCACATTCA	(GT) ₁₂	225	UP
	63105R	AATTGTCGTTTTTCCGTTCTG			
4	76181F	AGCACGTTTTGTCCCACTTG	(CT) ₁₀	218	UP, SL GAn: KT591168
	76181R	CAGAAGCTCTACTGGGGTACG			
5	125190F	ACGATCCTACTCCGGCTTTA	(CCGTG) ₄	222	UP
	125190R	CGTCGCGTTATGAGCTAGAAA			
6	204211F	CTGCGGAGGACGTGATTATT	(CTAT) ₁₆	267	-
	204211R	GGCAAAGGTCTGAGATGGAC			
7	205168F	GTGCTGCGGAGGACAATC	(CTAT) ₁₀	194	-
	205168R	GGTGGCAAAGGTTTGAGATG			
8	271200F	AAATGCCCAAGAGCTCGTTAT	(CA) ₁₃	226	UP, SL GAn: KT591169
	271200R	GCCGCCTGCTTCTGTAAAC			
9	303184F	CACGGAACGGTTACCAGAAG	(TGC) ₇	221	-
	303184R	GGGCAAACCTGGAAGTTGT			
10	326182F	GCGCGCAGACGTGTTAGTA	(TTG) ₇	222	-
	326182R	CAAAGAAATCGAAGAGGGTCTC			
11	401155F	GGGTACAAGTTGAAGCGATTT	(ACG) ₇	223	-
	401155R	TCTGCTGCACGATCTGTATG			
12	401187F	CCCGGACAGCACAGATTAATA	(TGAATG) ₅	230	-
	401187R	TCTTTCAAGCTAACCGTGCAT			
13	516192F	AAATGACCAAATCCGAAAGG	(ACC) ₇	221	UP, SL GAn: KT591170
	516192R	TTTTACGGCGGCTGTATCAC			
14	538199F	CTGCGGAGGACGTGATTATT	(CTAT) ₁₃	240	-
	538199R	GGCAAAGGTTTGAGATGGAC			
15	585164F	AATTCACGAGGCTGCATTTT	(AG) ₁₄	164	UP, SL GAn: KT591171
	585164R	CACGGATACGTAGGGAGTACG			
16	591177F	CTTGAAGGCCATTGGGAAAT	(TC) ₁₂	225	-
	591177R	TCCCCACAGTATTCCAGCAT			
17	638128F	ACGTTTTCTGTTTGTCCGTTG	(CGA) ₇	219	UP, SL GAn: KT591172
	638128R	GATTTTCCCCGCAAACTTC			
18	652240F	ACCTGGCGAACCTGATACAC	(GAGGAA) ₉	296	-
	652240R	CAAAAGAACACAAACGTCTCG			
19	668183F	TGCATTGTGTTTTATTTTATCAGC	(TGA) ₉	228	UP, SL GAn: KT591173
	668183R	TCGATATTCCAATCCAAACCA			
20	686148F	ATCCAGGCTAGTGCCAGAAC	(CAGA) ₅	188	-
	686148R	GAGAAGGGCTAACTCGCTCA			

Table S1. *Cont.*

No.	Locus	Primer Sequence	Repeated Motif	Sequence Size	Remarks
21	752140F	CCATTCTCCAATCAAGGAAAA	(CAGCT) ₇	237	-
	752140R	CAGAAATTTGCAAGGGGTCA			
22	807181F	ACCAAGAGAAGCGAGACCAA	(GCC) ₇	219	-
	807181R	TAGCAGTTGATGCCGTTTGA			
23	809183F	AGCCAGCTGAAGGAACACAC	(TCCCAT) ₄	228	UP
	809183R	ACGACGTTTCGAATCCACTC			
24	814124F	TTGTTTTTCAGCTGTCGAGGA	(TGT) ₇	221	-
	814124R	CCCGGCTTTTCAGGTATTACA			
25	853148F	AGTTTGCACGCCCTCTATTG	(TGTGT) ₄	222	-
	853148R	TTGCACACTCACGTTCTTCC			
26	914143F	TTGCCAAAAGTACTCCATTCTG	(CTCTA) ₄	221	-
	914143R	CATGGAAATCACAACAGAATTT			
27	916145F	CAGGTCAATTACCGCGAACT	(GTGAAA) ₃	222	-
	916145R	TTGCATCCGAAGAAAATGAA			
28	951157F	CGACCGTTCCTAACGTACTTG	(CTT) ₇	221	-
	951157R	GGTCAGAAGAAGGCGAACAG			
29	951171F	CGCGGCTAACTTTTGTCTTT	(GAT) ₇	223	UP, SL GAn: KT591174
	951171R	ACTTCCGCCCATTCATCTA			
30	959186F	GGGAAGAAGAGGAGCAGGAG	(AGA) ₆	221	-
	959186R	AACTTTCGTAGGCTGTCAAATG			
31	1008136F	TGGTTTTTAATCGATATTTTCATTGTC	(GAAGAT) ₄	232	-
	1008136R	TGTTTCACGTGGTACGATTCA			
32	1028155F	TTTTCTTCGCCCTTCCACTTC	(GAA) ₇	223	-
	1028155R	CCTGTGCACGAGAGATCCAT			
33	1043174F	GGGTGTGAGTTAGCATCAGC	(GA) ₁₁	223	-
	1043174R	CGTGATTCGACTGCACTTTT			
34	1109153F	CGATCGGCTTTCGTTATTTC	(AGG) ₇	220	-
	1109153R	CACTGTCACTTTCGCCCTCT			
35	1109178F	AGGGGTTTTCTTTTCGATGA	(AGG) ₇	220	-
	1109178R	CGAAGGTCGGCTTGAATAAA			
36	1158150F	GCGACTATGGAAGAATTTCTTAA	(GAT) ₆	220	-
	1158150R	TGTTTTTCAGCCTCGTCTTCA			
37	1171190F	GCACGAAAGTCCCGAAGATA	(ACA) ₇	222	-
	1171190R	TGAGGACATAGCCGTACTCG			
38	1192175F	ATCTCAATCCGATGGCTCTG	(CTGT) ₆	227	-
	1192175R	AAAAAGCGGTGTGGTGCTAT			
39	1444161F	ATCTCAATCCGATGGCTCTG	(GTCT) ₅	220	-
	1444161R	AGATCCGCAGTTCAAACGTC			
40	1539174F	TCGGCGAGATTTGAATTTTT	(TG) ₁₀	220	UP, SL GAn: KT591175
	1539174R	AGGATGTCCCACATTTGACG			
41	1627163F	CGAGATCGAGAATGAAACCAA	(CGCGG) ₄	223	UP
	1627163R	AACGAAGGCAAACGAGGAC			
42	1632148F	GACGATATGTGGGCAATTCA	(CAA) ₆	220	-
	1632148R	TATCTTCGACTTCGGCTTCG			

Table S1. *Cont.*

No.	Locus	Primer Sequence	Repeated Motif	Sequence Size	Remarks
43	1657165F 1657165R	TTGCACGACCACAAAAATGT ATCCGTTGAGAGAACGATGC	(CAA) ₆	220	UP
44	1744180F 1744180R	GCCCCCAAAGAAAAAGAAA ATCATGCCATGTCCCAACTT	(AC) ₁₁	223	UP
45	1866170F 1866170R	ACCATCAATTTCTGCAACCA GAGCGATACCTATGCACTCTATT	(TGTA) ₅	220	-
46	1949162F 1949162R	ATCTCAATCCGATGGCTCTG TGTGCCACGTTTAGATTTC	(TGTC) ₅	221	UP
47	1958160F 1958160R	CACAAACCTTCCTCCACGAC CGGCAGAGACAGACGTGAT	(CCT) ₇	223	-
48	1958168F 1958168R	CGCTTTGGCTTCCATAACTT TGCCGTCAAAGGAATATGTTT	(ACC) ₇	221	-
49	1958179F 1958179R	GCCACCTCCACTGTATCCTAA GGGGTGGTGGTTATGGTAATC	(TCC) ₇	221	UP
50	2030171F 2030171R	AAACTACCGGTTTGCCTGTG CAAGTTTGAATTCACAAACGAA	(TCA) ₁₀	231	-
51	2051159F 2051159R	CCCGTCACTGCAAATTGAT AAGGCGTCGTCGTCATTAAC	(TCC) ₁₂	237	UP
52	2051169F 2051169R	GTCGACCGTCATTTGTTCTT CGTTTTTCAGGCTCCATTCAT	(CGA) ₆	193	UP
53	2055128F 2055128R	GCCGAAATTCTTTGTTGCTG TGACGTTTGTAGCGATTCAA	(CA) ₁₁	222	-
54	2204176F 2204176R	ATATTCGCGAGTGACAGACG AATACGTTTCGAGCAAACAA	(AG) ₁₂	224	-
55	2204178F 2204178R	CGCGTCATTTAGCGAATTTT AGACGCAACACAACATTTGAA	(TGCC) ₅	220	-
56	2316127F 2316127R	GCAGCCTTCAAGACAGGATT TTGCCTTCCTACTTCCTCCA	(TGG) ₉	227	-
57	2441163F 2441163R	TTTCCAAACTCAAAATGCACTG TGTAATAAAATTAGCTCTACGTGTCA	(ATAC) ₅	222	-
58	2538170F 2538170R	CGGACGAGGTTCTGTCTAT ATCTCCACGGTCATGCTTTT	(TC) ₁₄	228	-
59	2547173F 2547173R	AATTGGGCCACGAAGTTTTT AACAACGCCAGAAGGAGAAA	(TC) ₁₄	200	UP
60	2715181F 2715181R	TATAAGGCGCACAGATTGTC GACCCAATCAACTTTAACCTG	(TGC) ₉	227	-
61	2733154F 2733154R	TAAACCAGTTTGTGCAATGG ATGAGAGCTAATGGCTACGG	(GGC) ₇	222	UP
62	2854160F 2854160R	CCGAATCCTCCTTTCCATC CGTATTCAGCTGGCTACTCCA	(CCG) ₆	220	
63	2888152F 2888152R	CGTGTGCAATTTTCTTTTTC CCAACCTAATCACTTCCCTAGTTT	(TC) ₁₇	235	UP
64	2983187F 2983187R	GCGGCATTAAACTCTACAGGA TCACCTATCGGTATAGCCTCAAA	(TCT) ₁₁	235	UP

Table S1. Cont.

No.	Locus	Primer Sequence	Repeated Motif	Sequence Size	Remarks
65	3014173F	ATGTGCGACTGAAAATTCTGG	(CGA) ₈	225	UP
	3014173R	GAGCAGATCAAAGCCCCTATAA			
66	3056155F	ACACAGAAGCCAACAGTAGCC	(AC) ₁₀	221	-
	3056155R	TATGGGAGTCACTTCGTCTGC			
67	3075173F	CGCGGAATATAAAGTCGTGTT	(GTG) ₇	221	UP
	3075173R	GAAAGTAATCACCGCGCTAAA			
68	3217182F	CCCTTCTTGTCTAGGGAGACC	(CAGA) ₅	203	-
	3217182R	GAGGTGAGAAGGGCTAACTCG			
69	3389169F	GTGCCAGGAACACGATTACAC	(AC) ₁₂	225	-
	3389169R	GTTCAAAAGGGGTCGGATATT			
70	3407159F	CATGCAAAAGCACGTATCACTT	(GTC) ₈	224	-
	3407159R	TAGACCGAGATTTGGGGATTTA			
71	3545149F	AAATGTTGCTATTACTGAAACACAAA	(AC) ₁₄	167	-
	3545149R	TGTAACATCGGAACCTATGTACGTTT			
72	3596176F	ATACACGGCCAGTTCTATGATTT	(TG) ₁₀	220	UP
	3596176R	ACTGAAAACGAACCTCTATCCAC			
73	3620155F	ATTGGAAATTTGAAGAGCCAAA	(GA) ₁₁	223	-
	3620155R	AAAACCTTAGAAGGCGTGGCTA			
74	3620160F	TCAAGAATCAGAACATGAAAGCA	(TC) ₁₀	221	-
	3620160R	AAAAACCAACTCGTCATAAACCA			
75	3620168F	GAAAAATGAGCAGAAGTTTTTCG	(AAG) ₆	219	-
	3620168R	CAAGACTCGTCTTTCATCCATCT			
76	3620171F	AAAGGGGAAAAAGATTGGAGAA	(AAG) ₇	224	-
	3620171R	ATTTTCCTATTGCGCTTCCTTT			

Table S2. Collecting information and life stage of *D. hopei* individuals used for NGS and marker utility test (CRW, Capture from Rotten Woods; LT, Light Trap; WI, Wild Individual; BI, Breeding Individual).

No.	Collecting Site	Collecting Date	Life Stage	No. of Individual	Collecting Method	Remarks
1	Korea Jeollabuk-do Wanjū	5 February 2013	Adult	1	CRW	WI (Template DNA for NGS)
	Yongjin-myeon					
	Hagae Maeul					
2	Korea Jeollabuk-do Wanjū	20 December 2008	Adult	8	CRW	WI
	Yongjin-myeon Gueok-ri	7 March 2015	Larva	12	CRW	
3	Korea Chungcheongnam-do Nonsan Yeonsan-myeon	No data	Adult	37	-	BI (emergence date: January 2013)
4	Japan Honshu Yamanashi Prefecture Nirasaki-shi	August 2012	Adult	6	LT	WI
5	China Guangxi Nanning	July 2012	Adult	17	LT	WI
6	China Shichuan Chengdu	July 2012	Adult	16	LT	WI

Table S3. Allele frequency of nine polymorphic microsatellite loci in regional populations of *Dorcus hopei*.

Microsatellite Locus	Allele Size (bp)	Guangxi China (n = 17)	Shichuan China (n = 16)	Wanju Korea (n = 20)	Nonsan Korea (n = 37)	Nirasaki Japan (n = 6)
10203	172	0.0882	0.0000	0.0250	0.0429	0.0000
10203	174	0.0000	0.0000	0.0500	0.0000	0.0000
10203	180	0.0000	0.0000	0.0750	0.0000	0.0000
10203	184	0.0000	0.0000	0.0500	0.0286	0.0000
10203	188	0.0294	0.0938	0.0000	0.2000	0.5000
10203	190	0.0882	0.1875	0.0000	0.0714	0.0000
10203	192	0.0294	0.1250	0.0000	0.0000	0.0000
10203	194	0.2059	0.1250	0.1750	0.0286	0.0000
10203	196	0.2059	0.1563	0.2750	0.1143	0.0000
10203	198	0.0882	0.2188	0.0000	0.1286	0.0000
10203	202	0.0882	0.0313	0.0750	0.0286	0.0000
10203	203	0.1176	0.0313	0.0250	0.0286	0.3000
10203	204	0.0588	0.0000	0.2500	0.3286	0.2000
10203	252	0.0000	0.0313	0.0000	0.0000	0.0000
76181	176	0.5000	0.5313	0.5000	0.5000	0.4167
76181	181	0.5000	0.4688	0.5000	0.5000	0.5833
271200	186	0.0294	0.1250	0.0000	0.0000	0.0000
271200	188	0.0294	0.0313	0.0000	0.0000	0.0000
271200	190	0.0000	0.0000	0.3000	0.1216	0.0000
271200	192	0.0000	0.0938	0.0000	0.0000	0.1000
271200	194	0.1176	0.0625	0.1000	0.0000	0.0000
271200	196	0.0294	0.0625	0.0750	0.3514	0.3000
271200	198	0.4412	0.1250	0.4750	0.4595	0.5000
271200	200	0.2941	0.4063	0.0000	0.0405	0.0000
271200	202	0.0294	0.0625	0.0000	0.0135	0.0000
271200	206	0.0000	0.0000	0.0500	0.0135	0.1000
271200	210	0.0000	0.0313	0.0000	0.0000	0.0000
271200	211	0.0294	0.0000	0.0000	0.0000	0.0000
516192	178	0.0588	0.1250	0.0000	0.1216	0.0000
516192	180	0.0294	0.0313	0.0000	0.0000	0.0000
516192	182	0.0882	0.0313	0.0000	0.0270	0.0000
516192	184	0.2941	0.4063	0.2750	0.3108	0.0000
516192	186	0.1471	0.1250	0.0000	0.0135	0.0000
516192	188	0.0000	0.0313	0.0500	0.0811	0.0000
516192	190	0.3529	0.2188	0.6750	0.4324	0.8750
516192	200	0.0000	0.0000	0.0000	0.0000	0.1250
516192	208	0.0000	0.0000	0.0000	0.0135	0.0000
516192	218	0.0000	0.0313	0.0000	0.0000	0.0000
516192	224	0.0294	0.0000	0.0000	0.0000	0.0000

Table S3. *Cont.*

Microsatellite Locus	Allele Size (bp)	Guangxi China (n = 17)	Shichuan China (n = 16)	Wanju Korea (n = 20)	Nonsan Korea (n = 37)	Nirasaki Japan (n = 6)
585164	122	0.0294	0.0625	0.0000	0.0000	0.0000
585164	160	0.5882	0.5938	0.3750	0.2973	0.1000
585164	162	0.3235	0.2813	0.2750	0.0676	0.1000
585164	164	0.0294	0.0313	0.0250	0.1892	0.4000
585164	170	0.0294	0.0000	0.0000	0.0000	0.0000
585164	174	0.0000	0.0313	0.2500	0.4459	0.4000
585164	176	0.0000	0.0000	0.0250	0.0000	0.0000
585164	208	0.0000	0.0000	0.0250	0.0000	0.0000
585164	214	0.0000	0.0000	0.0250	0.0000	0.0000
638128	171	0.0455	0.0000	0.0000	0.0000	0.0000
638128	177	0.0000	0.0000	0.0250	0.0000	0.0000
638128	179	0.0000	0.0313	0.0000	0.0000	0.0000
638128	183	0.0000	0.0938	0.0250	0.0000	0.0000
638128	219	0.0000	0.0313	0.0000	0.0000	0.0000
638128	225	0.0000	0.0000	0.0000	0.0417	0.0000
638128	227	0.7273	0.4688	0.7500	0.7917	0.6250
638128	229	0.0000	0.0000	0.0000	0.0000	0.2500
638128	230	0.0000	0.0313	0.0000	0.0000	0.0000
638128	231	0.0000	0.0000	0.0000	0.0139	0.0000
638128	243	0.0909	0.0625	0.0000	0.0000	0.0000
638128	244	0.0000	0.0000	0.0750	0.0000	0.0000
638128	245	0.0000	0.0000	0.1250	0.0556	0.0000
638128	247	0.1364	0.2500	0.0000	0.0000	0.1250
638128	251	0.0000	0.0313	0.0000	0.0972	0.0000
668183	148	0.0294	0.0333	0.0000	0.0000	0.0000
668183	160	0.0588	0.0000	0.2500	0.4722	0.6250
668183	162	0.1471	0.2000	0.0000	0.0139	0.1250
668183	164	0.5588	0.4333	0.2750	0.1528	0.0000
668183	166	0.0294	0.1000	0.0000	0.0000	0.0000
668183	168	0.0294	0.0333	0.0000	0.0000	0.0000
668183	174	0.0294	0.0333	0.0000	0.0000	0.0000
668183	176	0.0000	0.0000	0.0000	0.0278	0.0000
668183	180	0.0588	0.0000	0.3500	0.1806	0.1250
668183	182	0.0588	0.1667	0.1250	0.1528	0.1250
951171	164	0.0882	0.0333	0.1000	0.0676	0.0000
951171	166	0.0000	0.0333	0.0500	0.0000	0.0000
951171	170	0.9118	0.9333	0.8500	0.9324	1.0000

Table S3. Cont.

Microsatellite Locus	Allele Size (bp)	Guangxi China (<i>n</i> = 17)	Shichuan China (<i>n</i> = 16)	Wanju Korea (<i>n</i> = 20)	Nonsan Korea (<i>n</i> = 37)	Nirasaki Japan (<i>n</i> = 6)
1539174	164	0.0000	0.0625	0.0250	0.0286	0.0000
1539174	166	0.0625	0.0313	0.0000	0.0000	0.0000
1539174	168	0.3750	0.4375	0.1500	0.1571	0.3000
1539174	170	0.0938	0.0313	0.0250	0.0000	0.1000
1539174	172	0.1250	0.0938	0.1000	0.1143	0.0000
1539174	174	0.1250	0.0938	0.5500	0.5714	0.4000
1539174	176	0.0938	0.0938	0.0750	0.1143	0.0000
1539174	177	0.0313	0.0313	0.0750	0.0143	0.2000
1539174	178	0.0000	0.0938	0.0000	0.0000	0.0000
1539174	182	0.0625	0.0313	0.0000	0.0000	0.0000
1539174	186	0.0313	0.0000	0.0000	0.0000	0.0000