

Table S1. Details of the sampling sites and number of Korean spotted sleeper *Odontobutis interrupta*

Location	Code	Water system	N	Location
Oknyeodongcheon stream	SOD	Seomjingang River	23	35°39'43"N 127°09'09"E
Churyungcheon stream	SCR	Seomjingang River	21	35°26'05"N 126°56'02"E
Galdamcheon stream	SGD	Seomjingang River	24	35°31'35"N 127°09'21"E
Wonjucheon stream	HWJ	Hangang River	20	37°22'59"N 127°56'30"E
Dumicheon stream	HDM	Hangang River	20	37°41'39"N 127°40'06"E
Dalcheon stream	HDC	Hangang River	23	36°36'55"N 127°42'06"E
Jahocheon stream	NJH	Nakdonggang River	22	36°01'06"N 128°58'43"E
Migokcheon stream	NMG	Nakdonggang River	23	35°34'50"N 128°20'44"E
Gapcheon stream	GGP	Geumgang River	23	36°19'04"N 127°21'12"E
Nonsan Stream	GNS	Geumgang River	22	36°10'31"N 127°08'31"E
Bogangcheon stream	GBG	Geumgang River	23	36°48'22"N 127°36'59"E
Namcheon stream	HSNC	Hyeongsangang River	23	35°49'42"N 129°12'05"E

N: Number of samples.

Table S2. Summary of microsatellite screening and raw data of sequencing in *Odontobutis interrupta*.

Short reads	Total reads	Total read length (bp)
Raw data	4,075,232	1,083,799,779
Microsatellite search		
Contig		101068
Di-nucleotide		1282
Tri-nucleotide		2456
Tetra-nucleotide		1675
Penta-nucleotide		190
Hexa-nucleotide		50

Table S3. Details of microsatellite loci in *Odontobutis interrupta*

Locus	Primer sequence (5'→ 3')	Motif repeat	Product size (bp)	Dye	N (HDM=8, GBG=9, SGD=8)	N _A	H _O	H _E	PIC	Null allele	GenBank Accession no.
Oin3	F: TGTAACGACGGCCAGTAATTGAACCCTGTGAATGCC R: TGATGCCAATTGTACTCGCT	(CAT) ₂₄	274	PET	25	12	0.640	0.842	0.808	X	OQ656882
Oin27	F: TGTAACGACGGCCAGTGCCTGTATTTGAATTC TCG R: GGGCATCCGACGTAAACT	(TTCT) ₁₄	278	NED	25	9	0.760	0.867	0.832	X	OQ656883
Oin29	F: TGTAACGACGGCCAGTGTAGCTTGGGTGAATCAGGC R: CATATGCGACACAAGGTTGG	(GT) ₁₃	271	NED	25	5	0.760	0.675	0.622	X	OQ656884
Oin40	F: TGTAACGACGGCCAGTTTGTGTTTTAATTCTGGCTTTT R: CATCTCATGAATCACCAGGAA	(AATG) ₁₂	279	NED	25	11	0.840	0.888	0.857	X	OQ656885

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