Additional file 3. Characterisation of redesigned Primer set_B for *Hydrobia ulvae*

Locus	Corresponding A-primer clone name	Primer set	Repeat motif	Exp. allele size (bp) [†]	Primer sequence 5'–3' with 5' label or pigtail* (underlined)[‡]	$Pv3$ T_m (°C)Ψ	T_a (°C)	$MgCl_2$ (mM)	N	A	Allele range (bp)	H_O	H_E	HWE p-value
Hulv-01	66C08	B	GA(CA)$_3$(GACA)$_4$(GA)$_2$(CA)$_2$ (GA)$_{22}$CA(GA)$_7$CA(GA)$_4$CA (GA)$_4$ CA(GA)$_7$	230	F: HEX-GATCTGGGGCACACACAA R: (GTTTCTT)-CACAAATCGGGGAGAAGG	59.0 59.0	51.0	2.0	40	24	214 – 338	0.63	0.83	**
Hulv-02	67G01	B	Primers not redesigned due to difficulty in identifying new primer sites											
Hulv-03	67H10	B	(A)$_{23}$ & CTTACTA(CTTA)$_7$	172	F: HEX-GACTCGGGCACTCCACAGTAAA R: (GTTTCTT)-TCAATAGGGAAGGGGTGAGTCC	63.7 63.6	55.0	2.0	46	16	155 – 177	0.59	0.89	**
Hulv-04	68D02	B	(CA)$_{30}$AACA	172	F: 6-FAM-ACGGGTGTAACAACGACAAT R: (GTTTCTT)-GATCTGCAAGAGAGATGTAAGAACT	57.9 57.5	51.0	2.0	47	27	134 – 204	0.92	0.91	NS
Hulv-05	69C07	B	(GTTA)$_{21}$	235	F: 6-FAM-GATCGTAGGCTACTTACTACCACTG R: (GTTTCTT)-TCTGACCTGGTGTGTCATGT	57.8 57.4	51.0	2.0	44	21	174 – 306	0.59	0.93	**
Hulv-06	60F03	B	(CTAA)$_4$(CTCA)$_{12}$	217	F: HEX-CACAAAGCAACAGCTATAACTAA R: (GTTTCTT)-TGATGGTGTCGTACTGAATC	54.5 54.3	51.0	2.0	40	21	169 – 249	0.33	0.94	**
Hulv-07	69A07	B	(GTTA)$_9$	154	F: 6-FAM-CCGCACTACCAATGTAGC R: (GTTTCTT)-ATCAATAATCGGGCTTAAAAC	55.2 55.1	50.0	2.0	34	20	123 – 236	0.12	0.95	**

[†]Allele size in sequenced library source individual; [‡]Pigtail: GTTTCTT added to 5' end of reverse primer to reduce stutter bands [33].
Note that in all cases the 7 base pair pigtails have been incorporated in the allele sizes stated.
Ψ, Primers designed and melting temperature (T_m) calculated using PRIMER3 (*Pv3*) [32].
T_a annealing temperature; *N,* number of individuals successfully genotyped from the Dyfi estuary, Wales; *A,* number of alleles observed;
H_O, observed heterozygosity; H_E, expected heterozygosity.
**Locus deviates significantly from Hardy-Weinberg equilibrium (p<0.01), estimated using GENEPOP v3.4 [25].
NS, no significant deviation from Hardy-Weinberg equilibrium.