

Supporting Information for:

Riverscape properties contribute to the origin and structure of a hybrid zone in a Neotropical freshwater fish

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Table S1. Specimens of *Nematocharax venustus* analysed in the present study. The sample size used for each dataset is specified within parentheses.

Location	Latitude/Longitude	Sample size	Lot number
1	-14.832417/-40.103459	Morphology (3), COI (8), RADseq (5)	UESB-ATS
2	-14.810437/-40.160068	COI (15), RADseq (3)	UFBA 8232
3	-14.790126/-40.185068	COI (15), RADseq (2)	UFBA 8233
4	-14.760241/-40.085279	Morphology (14), COI (15), RADseq (5)	UFBA 8230
5	-14.725936/-40.128403	COI (15), RADseq (2)	UFBA 8234
6	-14.698243/-40.064739	Morphology (15), COI (15), RADseq (5)	UFBA 8231
7	-14.637319/-40.058303	Morphology (13), COI (14), RADseq (5)	UFBA 8236
8	-14.631389/-40.087025	COI (10), RADseq (4)	UFBA 8239
9	-14.625111/-40.096424	COI (15), RADseq (4)	UFBA 8237
10	-14.605031/-40.102650	COI (36), RADseq (4)	UFBA 7953 UFBA 7954 UFBA 8238
11	-14.581611/-40.007188	Morphology (15), COI (15), RADseq (5)	UFBA 8235
12	-14.579911/-40.074562	COI (10), RADseq (3)	UFBA 8240
13	-14.567653/-40.071957	COI (16), RADseq (3)	UFBA 8241
14	-14.465698/-39.964355	Morphology (14), COI (14), RADseq (5)	UFBA 8229

Table S2. Set of parameters defined in the params file to run ipyrad for the samples of *Nematocharax* analysed in this study.

hzdepth4trim15	## [0] [assembly_name]: Assembly name. Used to name output directories for assembly steps
/scratch/ebiodiv/ipyrad_paramfiles	## [1] [project_dir]: Project dir (made in curdir if not present)
	## [2] [raw_fastq_path]: Location of raw non-demultiplexed fastq files
	## [3] [barcodes_path]: Location of barcodes file
/scratch/ebiodiv/hz_samples/*.fastq	## [4] [sorted_fastq_path]: Location of demultiplexed/sorted fastq files
denovo	## [5] [assembly_method]: Assembly method (denovo, reference, denovo+reference, denovo-reference)
	## [6] [reference_sequence]: Location of reference sequence file
pairgbs	## [7] [datatype]: Datatype (see docs): rad, gbs, ddrad, etc.
GATC,	## [8] [restriction_overhang]: Restriction overhang (cut1,) or (cut1, cut2)
5	## [9] [max_low_qual_bases]: Max low quality base calls (Q<20) in a read
33	## [10] [phred_Qscore_offset]: phred Q score offset (33 is default and very standard)
6	## [11] [mindepth_statistical]: Min depth for statistical base calling
4	## [12] [mindepth_majrule]: Min depth for majority-rule base calling
10000	## [13] [maxdepth]: Max cluster depth within samples
0.85	## [14] [clust_threshold]: Clustering threshold for de novo assembly
0	## [15] [max_barcode_mismatch]: Max number of allowable mismatches in barcodes
2	## [16] [filter_adapters]: Filter for adapters/primers (1 or 2=strict)
35	## [17] [filter_min_trim_len]: Min length of reads after adapter trim
2	## [18] [max_alleles_consens]: Max alleles per site in consensus sequences
5, 5	## [19] [max_Ns_consens]: Max N's (uncalled bases) in consensus (R1, R2)
8, 8	## [20] [max_Hs_consens]: Max Hs (heterozygotes) in consensus (R1, R2)
38	## [21] [min_samples_locus]: Min # samples per locus for output
20, 20	## [22] [max_SNPs_locus]: Max # SNPs per locus (R1, R2)
8, 8	## [23] [max_Indels_locus]: Max # of indels per locus (R1, R2)
0.5	## [24] [max_shared_Hs_locus]: Max # heterozygous sites per locus (R1, R2)
0, 0, 0, 0	## [25] [trim_reads]: Trim raw read edges (R1>, <R1, R2>, <R2) (see docs)
0, 15, 15, 0	## [26] [trim_loci]: Trim locus edges (see docs) (R1>, <R1, R2>, <R2)
*	## [27] [output_formats]: Output formats (see docs)
	## [28] [pop_assign_file]: Path to population assignment file

Table S3. Final statistics summary provided by ipyrad for the 55 samples of *Nematocharax* analysed in this study.

Sample	Location	Raw reads	Reads passing filter	Total clusters	Hidepth clusters	Estimated heterozygosity	Error rate	Consensus reads	Locs in assembly
4492	1	3029783	2853938	1102144	99396	0.008864	0.012165	204348	1394
4496	1	3016158	2960948	1214938	97131	0.007771	0.009693	201000	1372
4486	1	1487502	1436624	767514	17838	0.006801	0.029065	66934	1082
4488	1	1419824	1384348	818925	12433	0.013683	0.032877	51393	1019
4490	1	1495312	1445689	869473	12991	0.008166	0.032094	52907	996
0055	2	1797460	1745765	1153129	13055	0.015819	0.027919	50261	1017
0047	2	2880936	2849357	1247125	81509	0.008282	0.010297	183463	1390
0054	2	3309161	3200790	1399900	96530	0.009139	0.010438	202654	1412
0062	3	1407863	1385155	754360	14792	0.009947	0.019801	59160	1025
0075	3	3529125	3204190	1453618	86414	0.019047	0.012149	191300	1356
0023	4	1721179	1627771	1216977	6560	0.0221	0.031499	26355	748
0021	4	1624958	1507297	1120543	5987	0.03744	0.035371	24354	680
0022	4	1722315	1578666	1220680	5422	0.03744	0.035371	21024	647
0030	4	3965936	3911642	1925149	101056	0.008567	0.010406	207137	1394
0016	4	3161120	3076834	1789974	45966	0.013217	0.01308	126359	1290
0088	5	3382494	3305639	1757471	65497	0.009089	0.013019	162591	1345
0090	5	3766705	3719931	1836556	96348	0.008016	0.010907	203146	1388
0039	6	1377159	1152244	819595	4878	0.01606	0.034051	20782	661
0040	6	1471330	1449633	846241	12110	0.015576	0.01973	51502	947
0037	6	3592298	3480415	1582183	97804	0.008792	0.010006	205854	1383
0041	6	1580093	1541606	1111400	7267	0.023388	0.032182	28961	801
0035	6	1552097	1502734	892461	14714	0.008583	0.030658	57526	1073
0106	7	1609869	1513144	1058579	7967	0.00624	0.032129	32456	835
0119	7	1939887	1838617	1511399	4861	0.011541	0.039968	16025	445
0114	7	2319533	2289721	1365484	25432	0.013331	0.015702	86368	1162
0111	7	1627397	1579114	1143657	7018	0.023388	0.032182	29359	770
0112	7	4399868	4290985	2376415	88973	0.008247	0.010441	196772	1401
0154	8	2087033	2044986	1571904	7515	0.014112	0.033355	28588	736
0152	8	1480716	1444914	1087804	5099	0.021938	0.032397	20865	596
0149	8	3138350	3078682	1495424	73989	0.010091	0.011384	174906	1363
0151	8	3525927	3465438	1374682	126350	0.007702	0.009878	239476	1428
0126	9	3674394	3546683	1697984	94275	0.014676	0.010333	197113	1404
0129	9	1903898	1825961	1227794	12309	0.014459	0.029249	49234	1002

0134	9	3045467	2867653	1412842	63898	0.010531	0.010848	153815	1285
0131	9	1462897	1432226	956225	8704	0.013876	0.029525	36853	910
4257	10	3223369	3167284	1227284	113213	0.007543	0.009619	225178	1403
4259	10	1746242	1678727	1136672	10638	0.025637	0.030747	43647	927
0141	10	4295922	4199321	2860574	35006	0.017106	0.015004	103299	1205
0136	10	1860062	1765519	1200804	11866	0.008166	0.032094	46436	1003
0103	11	1777535	1657260	1360677	4806	0.025297	0.038968	13335	397
0105	11	2011662	1966999	1331196	14037	0.013683	0.032877	53381	1010
0104	11	1676627	1644784	1141870	9266	0.008407	0.034944	38307	905
0091	11	3143114	3097347	1159749	116361	0.007702	0.009878	224303	1413
0099	11	3192521	3075724	1334833	92099	0.014676	0.010333	195602	1397
0164	12	3068932	2986707	2208103	17357	0.008407	0.034944	59726	1081
0166	12	1649607	1631308	973454	14211	0.012639	0.020593	56668	1006
0162	12	1759027	1727446	1053776	14795	0.013665	0.020827	57290	993
0172	13	3390285	3331276	1920631	51210	0.011574	0.01247	135834	1300
0178	13	2757970	2726982	1231138	71923	0.008306	0.010476	170509	1345
0173	13	2362959	2256902	1479969	20362	0.013683	0.032877	71423	1152
0012	14	1670873	1586617	1202549	6014	0.013704	0.032813	23601	688
0015	14	1427049	1381155	1021498	5537	0.03744	0.035371	22939	636
0014	14	1590624	1560080	1093188	8527	0.008166	0.032094	35401	862
0001	14	3227816	3175439	1547827	78719	0.008262	0.010656	177044	1342
0005	14	3531690	3431759	1777854	75721	0.014676	0.010333	173323	1376

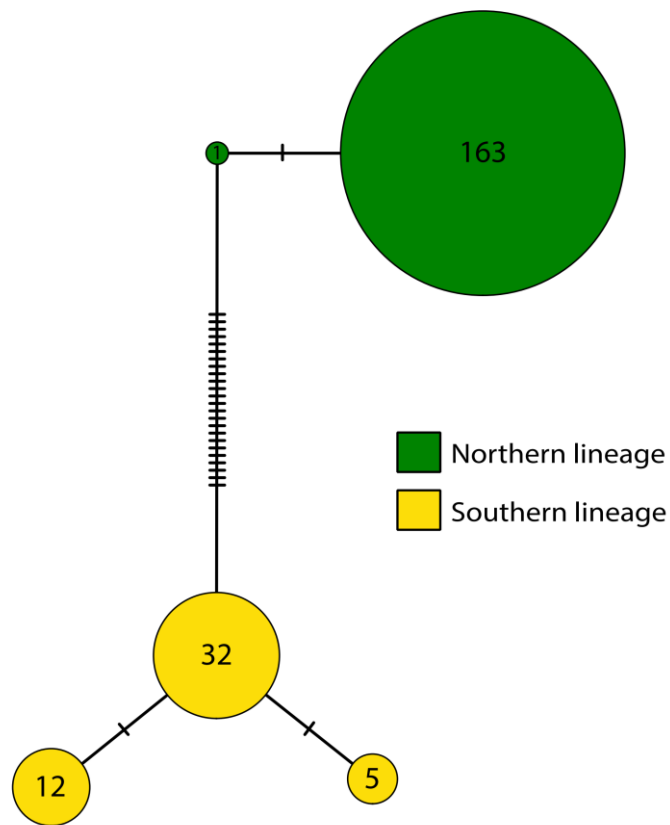


Fig. S1. Haplotype network for the cytochrome c oxidase subunit I (COI) gene including a total of 213 sequences with 650 base pairs (bp) each. The colors of the mitochondrial lineages correspond to those shown on the map in Fig. 1. Each circle corresponds to a haplotype and the size of the circles is proportional to the number of individuals (detailed within each circle) that share the same haplotype. Dashes between haplotypes represent mutational steps.

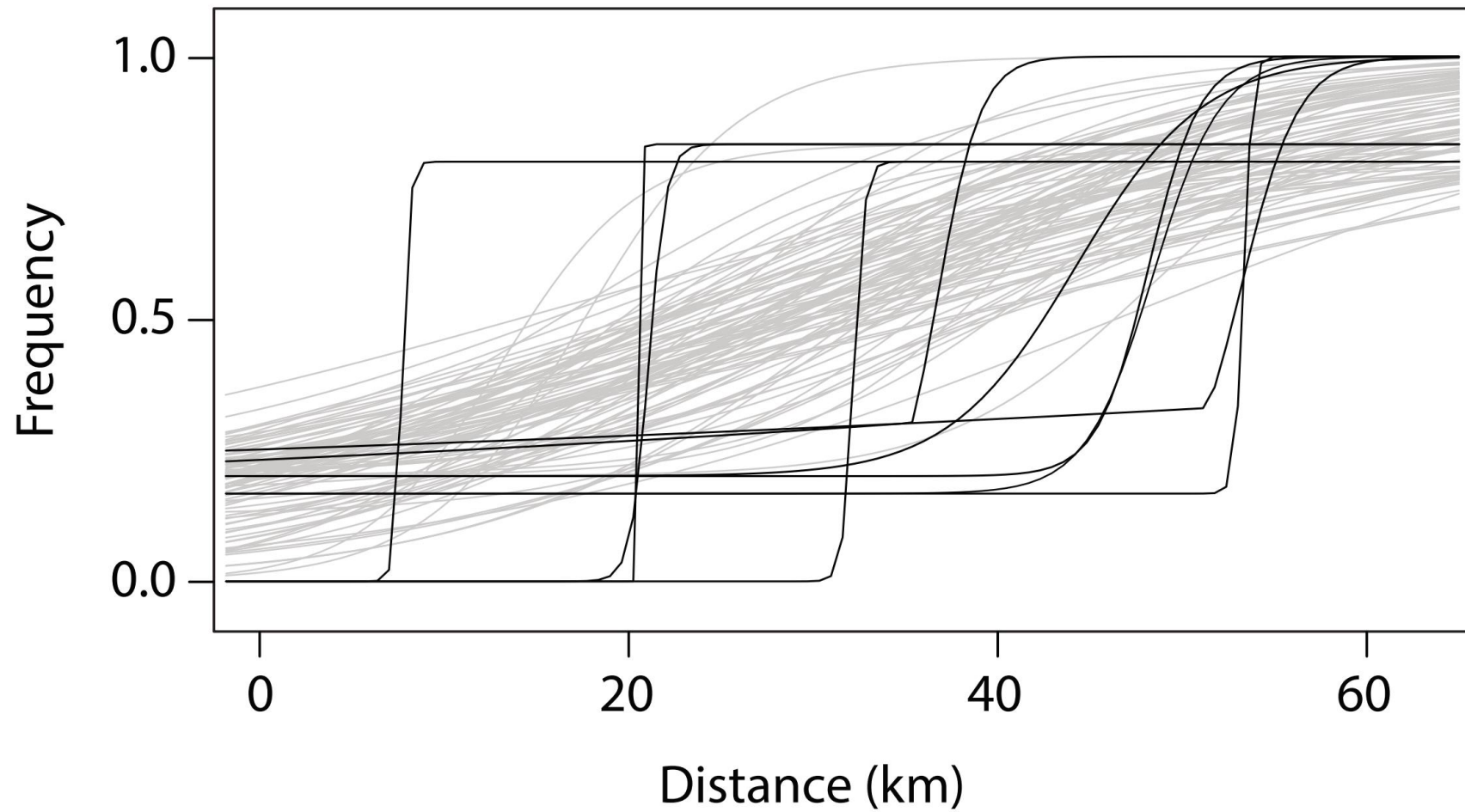


Fig. S2. Plots of the maximum likelihood clines estimated with HZAR for the 99 putatively unlinked SNPs (i.e., one SNP per locus), as detailed in the methods). The stepped clines are highlighted in black.

Table S4. Cline parameter values (centre and width, in kilometers) and confidence intervals estimated by HZAR from the best-fitting model for each of the 99 SNPs markers presented in Fig. S2. SNPs that showed stepped clines in Fig. S2 are indicated by an asterisk.

SNP	Centre	Width
SNP10002	33.27 (13.73–55.53)	104.26 (50.47–123.00)
SNP1009	29.47 (9.96–47.23)	55.19 (11.84–123.00)
SNP10111	21.69 (-2.56–43.20)	90.28 (43.06–123.00)
SNP10215	43.18 (26.08–68.14)	42.03 (0.01–123.00)
SNP10230	13.92 (-27.98–39.78)	68.16 (0.01–123.00)
SNP1042	44.41 (29.04–81.51)	45.99 (0.00–122.99)
SNP10473	29.75 (14.29–45.02)	48.48 (0.39–123.00)
SNP10608	25.75 (2.76–48.30)	102.34 (49.13–123.00)
SNP10618*	48.33 (32.43–88.78)	7.27 (0.00–123.00)
SNP10641	34.08 (18.28–52.91)	46.63 (1.11–123.00)
SNP10754	21.78 (2.75–38.63)	64.00 (33.21–123.00)
SNP10821*	53.24 (27.05–72.98)	0.91 (0.00–123.00)
SNP11000	34.51 (19.44–51.65)	46.26 (11.34–122.99)
SNP11225	25.75 (4.61–46.68)	119.58 (55.76–123.00)
SNP1131	43.44 (31.09–69.49)	29.69 (0.00–123.00)
SNP1255	38.09 (22.92–52.88)	34.46 (0.36–118.23)
SNP1289	27.36 (5.25–50.71)	89.90 (44.15–123.00)
SNP1393	29.64 (18.54–43.16)	40.52 (16.23–102.57)
SNP155	27.77 (6.29–51.12)	95.40 (46.53–123.00)
SNP1666	44.93 (28.12–83.69)	59.16 (0.00–123.00)
SNP18	24.27 (9.62–39.51)	40.10 (7.38–122.99)
SNP1816	16.53 (-6.77–30.11)	29.03 (0.31–123.00)
SNP1884	37.56 (22.50–62.97)	54.60 (0.01–123.00)
SNP1891	17.67 (-15.09–30.89)	30.45 (0.04–123.00)
SNP1964	27.98 (11.45–47.29)	68.46 (36.38–123.00)
SNP1993	13.02 (0.09–22.54)	14.91 (0.00–69.44)
SNP2042	19.94 (-4.06–37.61)	94.21 (47.79–123.00)
SNP2131	29.95 (8.88–53.05)	77.74 (38.56–123.00)
SNP2268	32.78 (21.64–43.58)	39.86 (21.53–86.50)
SNP2279	25.06 (6.62–45.50)	72.52 (37.31–123.00)
SNP2307	46.37 (31.26–78.65)	71.42 (36.76–123.00)
SNP2504	32.31 (16.75–56.69)	47.65 (5.82–123.00)
SNP2820	26.13 (11.78–44.24)	38.27 (0.02–116.82)
SNP2984	34.34 (19.18–60.47)	72.34 (37.62–123.00)
SNP3065	21.96 (-1.41–44.47)	94.09 (44.07–123.00)
SNP3103	33.70 (13.55–56.87)	106.04 (51.67–123.00)
SNP3310	26.62 (16.33–37.47)	45.32 (26.15–97.24)
SNP3836	16.13 (-2.74–29.00)	47.25 (23.62–123.00)
SNP3858	36.79 (13.06–61.09)	122.97 (57.14–123.00)
SNP4133	34.00 (14.82–56.63)	103.20 (51.22–123.00)
SNP4213	19.47 (-0.08–35.99)	60.99 (31.19–123.00)
SNP4304	47.79 (36.52–62.82)	21.53 (0.00–90.30)
SNP4357	34.80 (22.94–48.81)	43.05 (16.59–116.60)
SNP4424	24.93 (14.16–35.68)	46.89 (27.25–96.00)
SNP4511	20.85 (1.56–38.01)	64.37 (32.79–123.00)
SNP4617	26.99 (7.30–47.45)	85.95 (43.78–123.00)
SNP4696	34.74 (18.21–59.87)	50.63 (0.36–123.00)
SNP5002	21.62 (-2.26–40.59)	50.81 (0.01–123.00)
SNP5009	21.65 (8.33–35.55)	52.91 (28.89–123.00)
SNP5178	23.74 (4.49–44.47)	84.89 (42.26–123.00)
SNP5182*	53.12 (42.79–60.52)	6.13 (2.10–41.68)

SNP5421*	7.65 (0.03–12.62)	0.80 (0.00–27.23)
SNP5431*	44.16 (22.65–62.70)	13.75 (0.00–123.00)
SNP5485	29.15 (15.37–44.09)	78.21 (45.62–123.00)
SNP5604	29.80 (9.17–53.27)	92.39 (45.63–123.00)
SNP5808	37.75 (25.38–50.62)	32.83 (0.29–104.49)
SNP5884	26.92 (8.68–49.24)	55.93 (15.18–123.00)
SNP6013	38.42 (21.45–55.82)	35.89 (0.03–122.99)
SNP604	29.42 (8.44–50.53)	122.99 (59.12–123.00)
SNP6056	39.51 (19.60–64.46)	85.30 (43.52–123.00)
SNP6085	25.63 (15.14–36.30)	29.35 (8.75–67.44)
SNP6140	17.52 (-8.60–36.79)	77.66 (36.62–123.00)
SNP6180	32.04 (9.80–54.83)	122.94 (60.43–123.00)
SNP6269	21.16 (-5.30–39.67)	41.35 (0.00–123.00)
SNP6293	38.19 (26.09–59.22)	54.48 (29.87–123.00)
SNP6402	33.27 (17.59–51.92)	53.34 (22.40–123.00)
SNP6969	31.71 (18.10–50.23)	37.54 (14.80–116.78)
SNP7047	28.09 (11.24–46.70)	71.05 (38.67–123.00)
SNP7101*	48.10 (28.80–65.46)	5.51 (0.00–123.00)
SNP7355	29.87 (7.39–52.01)	122.98 (56.74–123.00)
SNP7402	40.47 (31.75–48.67)	22.90 (0.20–59.05)
SNP7510	29.59 (7.64–51.76)	100.59 (50.23–123.00)
SNP7584	20.09 (-12.88–43.67)	46.13 (0.14–123.00)
SNP7918	32.06 (23.03–43.08)	38.56 (21.90–77.98)
SNP794	21.26 (-4.19–44.24)	97.96 (44.16–123.00)
SNP7969*	20.56 (12.66–21.64)	0.18 (0.00–28.70)
SNP8146*	32.11 (21.73–42.93)	1.13 (0.00–50.82)
SNP8183	17.05 (-15.27–35.39)	58.22 (0.60–123.00)
SNP8208	28.72 (7.71–49.93)	117.27 (55.67–123.00)
SNP8274	33.74 (18.24–49.16)	46.21 (0.45–122.99)
SNP8437	31.00 (12.82–54.18)	88.67 (44.92–123.00)
SNP8486	24.84 (6.57–42.63)	70.33 (36.98–123.00)
SNP8577	24.68 (-0.38–49.24)	92.86 (42.59–123.00)
SNP8585	17.53 (11.70–24.67)	17.09 (8.03–39.12)
SNP8598	22.00 (8.25–38.75)	62.20 (32.98–123.00)
SNP8842*	21.00 (12.72–33.24)	1.89 (0.00–96.26)
SNP8879	31.25 (19.63–46.01)	34.42 (11.75–114.57)
SNP8997	31.60 (20.03–49.09)	55.65 (30.23–123.00)
SNP921	23.99 (8.35–41.48)	45.49 (12.46–123.00)
SNP9363	16.97 (-15.59–47.33)	49.47 (0.00–123.00)
SNP9427	23.08 (2.06–40.36)	70.53 (35.87–123.00)
SNP9583	35.75 (19.57–56.59)	35.86 (0.45–123.00)
SNP9673	36.14 (20.68–55.39)	68.76 (37.69–123.00)
SNP97	30.00 (16.21–48.85)	60.88 (33.01–123.00)
SNP9725	20.31 (1.56–34.44)	65.30 (35.70–123.00)
SNP9753	38.99 (25.69–56.82)	57.39 (32.00–123.00)
SNP9765	37.34 (15.59–59.30)	122.86 (57.08–123.00)
SNP991*	36.70 (31.44–43.62)	4.58 (0.65–36.76)
SNP9961	25.82 (2.71–45.08)	78.19 (40.55–123.00)
