Genomic signatures of local adaptation to the degree of environmental predictability in rotifers

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Supplementary Method S1: Definition of standard conditions.

Clones were maintained in 15 mL stock cultures at 12 g L^{-1} salinity, 20ºC and *Tetraselmis suecica* as food.

Supplementary Method S2: Protocol to grow the clone cultures in order to obtain enough biomass for DNA extraction.

The clone was allowed to grow under the standard conditions specified above for 15 days, starting from low density stock culture. During the first 5 days, rotifers were kept in a flask containing 200 mL of culture medium and, after that, they were transferred to eight bottles containing 1.5 L of culture medium. On day 10, when >10,000 individuals were reached in each bottle, rotifers were filtered out through a 30-µm Nytal mesh sieve and released on saline water 12 g L^{-1} for 24 hours in order to purge the rotifer's digestive tracts and thus minimize contamination with microalgae DNA. Finally, the animals were concentrated by filtering again with a new 30-µm Nytal mesh sieve to obtain the biomass needed for genomic DNA extraction.

Supplementary Data S1: File with the options provided to Maker2 with permissive settings.

Supplementary Table S1: Number of SNPs shared between each of the variables studied in Bayenv and the SNPs found to be candidates for diversifying selection (Bayescan). No SNPs candidate for being under balancing selection was also associated to any other variable.

	Diversifying selection	***Propensity for sex***	***Hatching fraction***	***Environmental predictability***	***Hydroperiod length***	***Salinity***
Diversifying selection	12					
Propensity for sex	1	37				
Hatching fraction	2	6	38			
Environmental predictability	0	10	4	34		
Hydroperiod length	0	4	7	6	39	
Salinity	1	2	10	2	5	39

Supplementary Table S2: Number of genes detected to be physically associated to SNPs under selection. Results for (1) three window sizes around each SNP and (2) three approaches to detect selection are reported.

Approach	Type of selection/ Associated variable	Number of SNP	Window size		
			0 Kb	2.5 Kb	5 Kb
BayeScan	**Balancing**	81	39	144	199
	Diversifying	12	12	37	49
Bayenv	**Propensity for sex**	37	34	95	139
	Hatching fraction	38	35	94	127
	Predictability	34	27	78	119
	Hydroperiod	39	28	80	119
	Salinity	39	27	72	114
GenABEL	**Propensity for sex**	1	1	1	1
Overall		261	164	483	696

Supplementary Table S3: Significantly overrepresented gene ontologies (GO) in the genes associated to the variables studied (Fisher's exact test, two- tailed, false discovery rate (FDR) < 0.05).

Variable **GO ID**	**GO Category**	**GO Name**	**FDR**	***p*-value**
Hatching fraction				
GO:0004871	MOLECULAR FUNCTION	signal transducer activity	2.80E-02	3.90E-06
GO:0005488	MOLECULAR FUNCTION	binding	1.08E-07	1.50E-11
GO:0097159	MOLECULAR FUNCTION	organic cyclic compound binding	3.60E-04	1.50E-07
GO:1901363	MOLECULAR FUNCTION	heterocyclic compound binding	3.60E-04	1.49E-07
GO:0005524	MOLECULAR FUNCTION	ATP binding	6.29E-04	5.57E-07
GO:0030554	MOLECULAR FUNCTION	adenyl nucleotide binding	6.29E-04	6.13E-07
GO:0043168	MOLECULAR FUNCTION	anion binding	6.29E-04	4.64E-07
GO:0032559	MOLECULAR FUNCTION	adenyl ribonucleotide binding	6.29E-04	6.09E-07
GO:0001882	MOLECULAR FUNCTION	nucleoside binding	1.35E-03	2.61E-06
GO:0001883	MOLECULAR FUNCTION	purine nucleoside binding	1.35E-03	2.49E-06
GO:0017076	MOLECULAR FUNCTION	purine nucleotide binding	1.35E-03	2.63E-06
Environmental predictability				
GO:0035639	MOLECULAR FUNCTION	purine ribonucleoside triphosphate binding	1.35E-03	2.37E-06
GO:0032555	MOLECULAR FUNCTION	purine ribonucleotide binding	1.35E-03	2.55E-06
GO:0032550	MOLECULAR FUNCTION	purine ribonucleoside binding	1.35E-03	2.48E-06
GO:0032549	MOLECULAR FUNCTION	ribonucleoside binding	1.35E-03	2.58E-06
GO:0032553	MOLECULAR FUNCTION	ribonucleotide binding	1.35E-03	2.83E-06
GO:0097367	MOLECULAR FUNCTION	carbohydrate derivative binding	2.03E-03	4.68E-06
GO:0043167	MOLECULAR FUNCTION	ion binding	2.03E-03	4.82E-06
GO:1901265	MOLECULAR FUNCTION	nucleoside phosphate binding	5.17E-03	1.37E-05
GO:0000166	MOLECULAR FUNCTION	nucleotide binding	5.17E-03	1.37E-05
GO:0036094	MOLECULAR FUNCTION	small molecule binding	5.77E-03	1.61E-05
GO:0003676	MOLECULAR FUNCTION	nucleic acid binding	7.15E-03	2.09E-05
Propensity for sex				
GO:0005488	MOLECULAR FUNCTION	binding	0.01	2.36E-06
O:0050794	BIOLOGICAL PROCESS	regulation of cellular process	0.01	3.08E-06
Salinity				
GO:0050789	BIOLOGICAL PROCESS	regulation of biological process	0.01	3.61E-06
GO:0065007	BIOLOGICAL PROCESS	biological regulation	0.01	6.83E-06

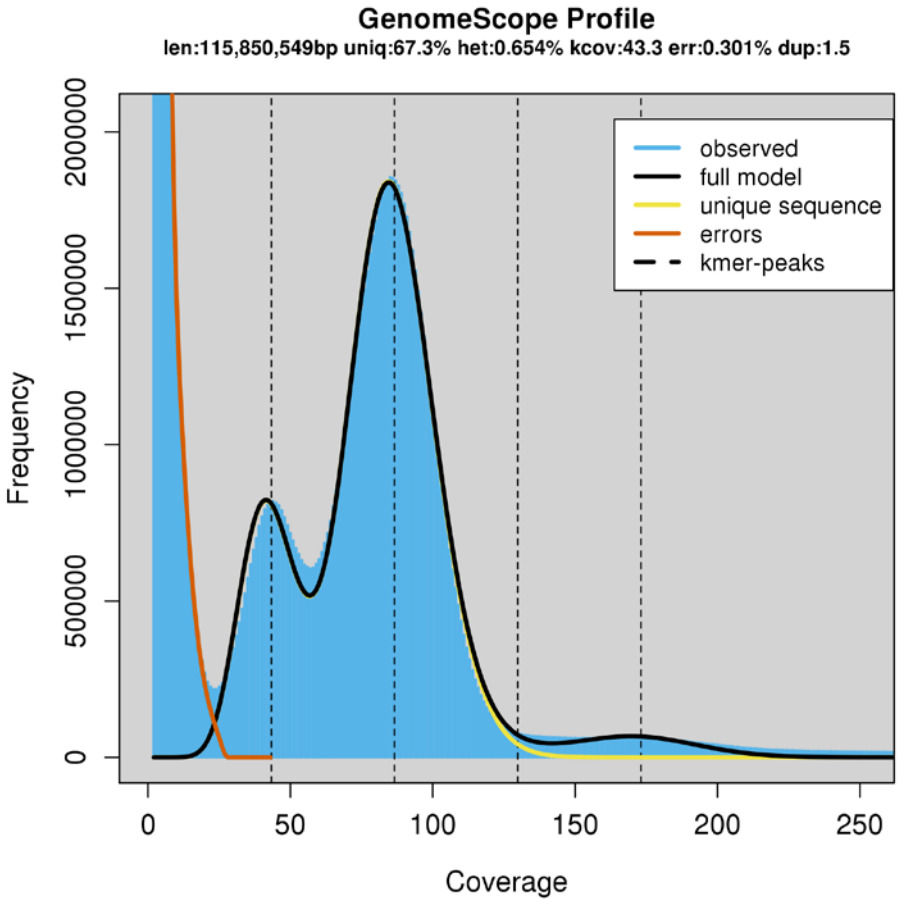
Supplementary Table S4: Transcriptomes used in the Brachionus plicatilis genome assembly from congeneric species.

Species	**Accession number**	**Reference**
B. manjavacas	GARS01000001-GARS01014244	Welch et al. (2014)
B. calyciflorus	GACL00000000.1 and GACQ00000000.1	Hanson et al. (2013)
B. koreanus	GBXV00000000	Lee et al. (2015)

Supplementary Data S2: File with the options provided to Maker2 with more stringent settings.

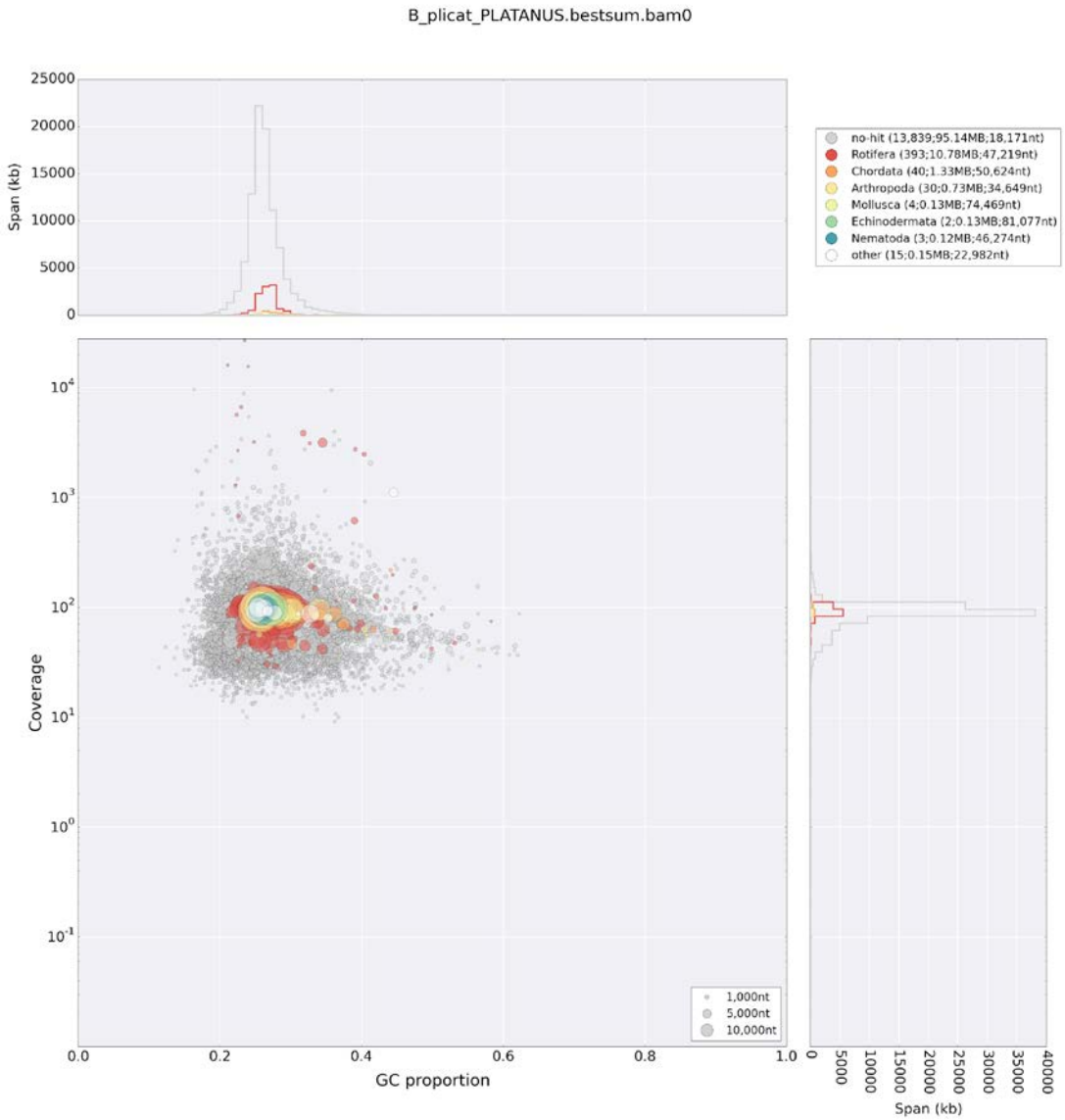
Supplementary Data S3: Ipython notebook used to quality filter the SNPs.

Supplementary Figure S1: K-mer plot and statistics for filtered and error-corrected raw data obtained from genome sequencing (k-mer length= 21).

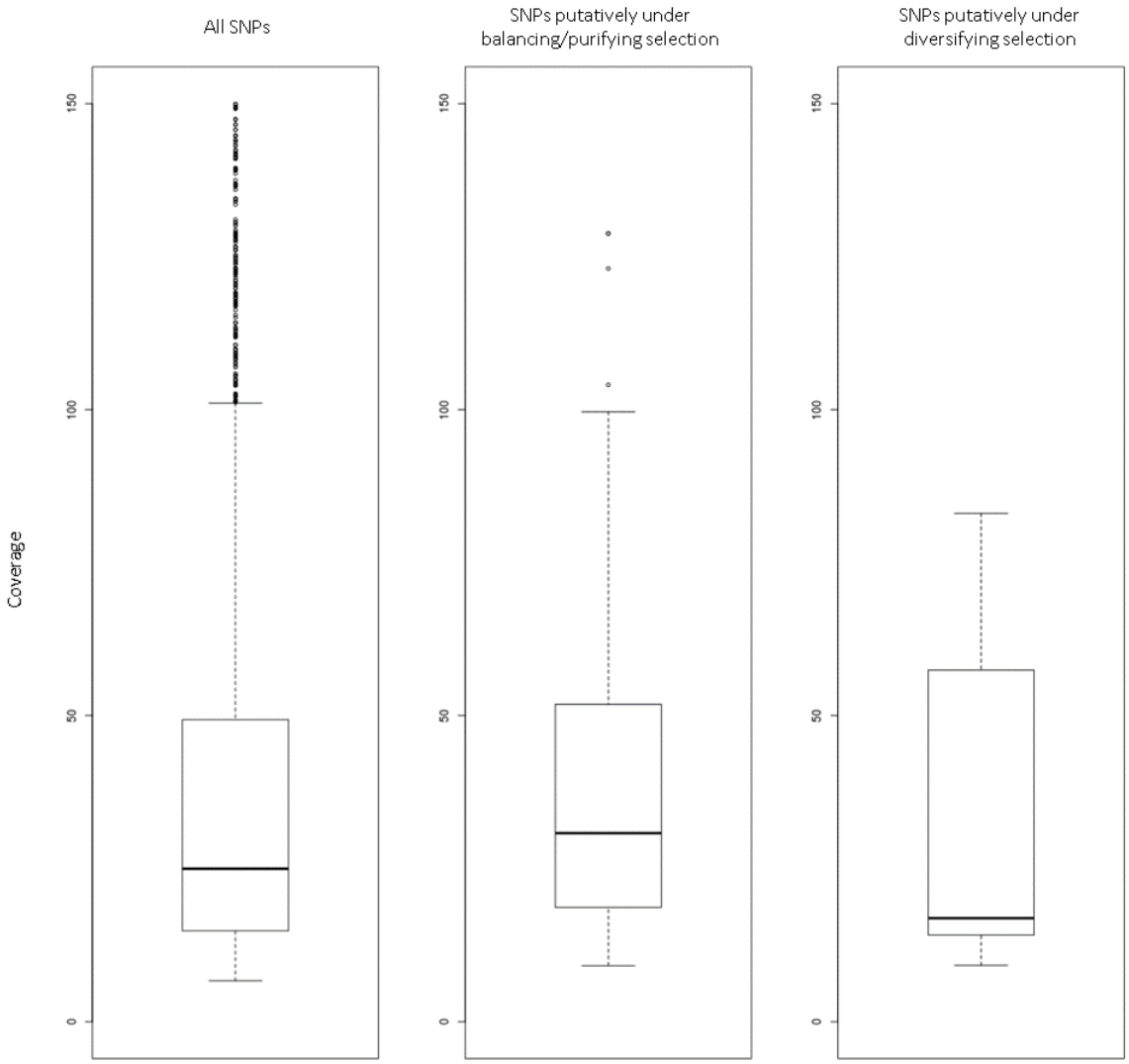


Property	min	max
Heterozygosity	0.653566%	0.658388%
Genome Haploid Length	115,775,183 bp	115,850,549 bp
Genome Repeat Length	37,904,922 bp	37,929,597 bp
Genome Unique Length	77,870,261 bp	77,920,952 bp
Model Fit	90.069%	93.868%
Read Error Rate	0.301464%	0.301464%

Supplementary Figure S2: Blobplot of the draft genome of *Brachionus plicatilis* based on G+C content and contig coverage.



Supplementary Figure S3: Boxplots showing the distribution of the coverage of the SNPs when: a) all SNPs were considered; b) Only those SNPs putatively under balancing/purifying selection or c) Only those that are putatively under diversifying selection.



Supplementary Figure S4: Within population linkage disequilibrium analysis between each pair of SNPs. X axis represents the genetic distance and Y axis represents the R^2. This analysis was performed using Plink (for references, see the main body).