Seascape genetics in a polychaete worm: disentangling the roles of a biogeographic barrier and environmental factors

Table S1. Sampling sites, GPS coordinates, and the number of specimens used in DNA extraction. The abbreviation for each location is in parentheses. NE- Northeast, SE-Southeast.

Sampling location	State (Region)	Latitude	Longitude	Date of Collection	# Specimens
Sabiaguaba (SBP)	Ceará (NE)	-3.7687	-38.4358	Jul 2017	9
Prado (PAP)	Alagoas (NE)	-9.6769	-35.7515	Jan 2019	5
Gamboa (GBP)	Espírito Santo (SE)	-20.8887	-40.7655	Jun 2018	9
Itaoca (ItaP)	Espírito Santo (SE)	-20.9050	-40.7770	Jun 2018	10
Prainha (PP)	Rio de Janeiro (SE)	-23.1482	-44.6955	Feb 2019	6
Jabaquara (JP)	Rio de Janeiro (SE)	-23.2112	-44.7141	Apr 2017 and Feb 2019	2
Praia Dura (DuP)	São Paulo (SE)	-23.4945	-45.1654	Jan 2018	10
Araçá (AP)	São Paulo (SE)	-23.8175	-45.4067	Jan 2017 and Feb 2019	10

Table S2. Variable selection results based on Factorial Analysis.

	factor1	factor2	factor3	factor4	factor5	factor6
Bathymetry	0.04	0.19	0.01	0.09	0.63	0.03
East/West aspect	0.1	0.01	0.06	0	0.04	0.03
North/South Aspect	0.01	0.04	0.14	0	0.05	0.01
Plan curvature	0.01	0.03	0.04	0.12	0.06	0.96
Profile curvature	0.01	0	0.01	0.98	0.07	0.16
Distance to shore	0.1	0.09	0.07	0.01	0.71	0.04

Bathymetric slope	0.01	0.08	0.15	0.05	0.09	0.29
Concavity	0	0.02	0.02	0.84	0.02	0.54
Sea surface salinity (annual mean)	0.68	0.48	0.06	0.03	0.46	0.01
Sea surface salinity (monthly minimum)	0.34	0.85	0.04	0.01	0.41	0.02
Sea surface salinity (monthly maximum)	0.84	0.06	0.17	0.03	0.35	0.03
Sea surface salinity (annual range)	0.16	0.95	0.14	0.01	0.23	0.05
Sea surface salinity (annual variance)	0.05	0.83	0.04	0.01	0.02	0.04
Sea surface temperature (annual mean)	0.79	0.07	0.5	0.04	0.23	0.07
Sea surface temperature (coldest ice-free month)	0.75	0.06	0.59	0.03	0.2	0.07
Sea surface temperature (warmest ice-free month)	0.82	0.06	0.37	0.04	0.25	0.07
Sea surface temperature (range)	0.27	0.03	0.96	0	0.03	0.07
Sea surface temperature (variance)	0.23	0.04	0.9	0	0.16	0.07
SS loadings	3.35	2.611	2.557	1.705	1.659	1.36
Proportion variation	0.186	0.145	0.142	0.095	0.092	0.076

Cumulative variation	0.186	0.331	0.473	0.568	0.66	0.736

Table S3. Initial parameter estimates for cline model construction on Hzar package. Types of scaling - fixed: model with minimum and maximum values fixed to the minimum and maximum observed mean values of data; none: model with fixed minimum value 0 and maximum value 1; free: model with the minimum and maximum value as free parameters. Types of tails – none: model with no exponential tails; right: A model with just one exponential tail on the right; left: model with just one exponential tail on the left; mirror: model with two exponential tails mirrored about the cline center; both: model with two tails with independent parameters.

Model Name	Scaling	Tails
Model 1	fixed	none
Model 2	fixed	right
Model 3	fixed	left
Model 4	fixed	mirror
Model 5	fixed	both
Model 6	none	none
Model 7	none	right
Model 8	none	left
Model 9	none	mirror
Model 10	none	both
Model 11	free	none
Model 12	free	right
Model 13	free	left
Model 14	free	mirror
Model 15	free	both
Null model		

Table S4. Environmental variables used in Principal Component Analyses of *Perinereis ponteni* distribution from Bio-Oracle and World Clim databases.

Bio-Oracle Environmental Variables	
Layer Code	**Variable Name**
BO_chlomax	Chlorophyll A (maximum)
BO_chlomean	Chlorophyll A (mean)

BO_chlomin	Chlorophyll A (minimum)
BO_chlorange	Chlorophyll A (range)
BO_nitrate	Nitrate
BO_ph	pH
BO_sstrange	Sea surface temperature (range)
BO2_carbonphytomax_bdmin	Carbon phytoplankton biomass (maximum at min depth)
BO2_carbonphytomean_bdmin	Carbon phytoplankton biomass (mean at mean depth)
BO2_carbonphytorange_bdmin	Carbon phytoplankton biomass (range at min depth)
BO2_chlomin_bdmin	Chlorophyll concentration (minimum at min depth)
BO2_chlomax_bdmin	Chlorophyll concentration (maximum at min depth)
BO2_chlorange_bdmin	Chlorophyll concentration (range at min depth)
BO2_dissoxltmax_bdmin	Dissolved oxygen concentration (longterm max at min depth)
BO2_dissoxltmin_bdmin	Dissolved oxygen concentration (longterm min at min depth)
BO2_dissoxmax_bdmin	Dissolved oxygen concentration (maximum at min depth)
BO2_dissoxmean_bdmin	Dissolved oxygen concentration (mean at min depth)
BO2_dissoxmin_bdmin	Dissolved oxygen concentration (minimum at min depth)
BO2_dissoxrange_bdmin	Dissolved oxygen concentration (range at min depth)
BO2_nitratemax_bdmin	Nitrate concentration (maximum at min depth)
BO2_nitraterange_bdmin	Nitrate concentration (range at min depth)
BO2_salinitymean_ss	Sea surface salinity (mean)
BO2_temprange_bdmin	Sea water temperature (range at min depth)
BO2_temprange_ss	BO2_temprange_ss
World Clim Biological variables	
Layer Code	**Environmental Variable**
bio1	Annual Mean Temperature
bio2	Mean Diurnal Range (Mean of monthly (max temp - min temp))

bio3	Isothermality (BIO2/BIO7) (×100)
bio4	Temperature Seasonality (standard deviation ×100)
bio5	Max Temperature of Warmest Month
bio6	Min Temperature of Coldest Month
bio7	Temperature Annual Range (BIO5-BIO6)
bio8	Mean Temperature of Wettest Quarter
bio9	Mean Temperature of Driest Quarter
bio10	Mean Temperature of Warmest Quarter
bio11	Mean Temperature of Coldest Quarter
bio12	Annual Precipitation
bio13	Precipitation of Wettest Month
bio14	Precipitation of Driest Month
bio15	Precipitation Seasonality (Coefficient of Variation)
bio16	Precipitation of Wettest Quarter
bio17	Precipitation of Driest Quarter
bio18	Precipitation of Warmest Quarter
bio19	Precipitation of Coldest Quarter

Table S5. Occurrence sites for *Perinereis ponteni* as available in publications from Web of Science® database.

Sampling location, state	**Latitude**	**Longitude**	**Source of record**
Paracuru, Ceará	-3.399704	-39.013765	Sousa, 2006 - Dissertation
Salinópolis, Pará	-0.697331	-47.370125	Chagas et al., 2018
Fortaleza, Ceará	-3.768708	-38.43586	Present study
Barra de Camaratuba, Paraíba	-6.603344	-34.964503	Paiva et al., 2019
Baía da Traição, Paraíba	-6.688583	-34.930508	Paiva et al., 2019
Tabatinga, Paraíba	-7.355767	-34.799122	Paiva et al., 2019

Tambaba, Paraíba	-7.366747	-34.797472	Paiva et al., 2019
Pina, Pernambuco	-8.089806	-34.879558	Paiva et al., 2019
Maceio, Alagoas	-9.676867	-35.751568	Present study
São Francisco do Conde, Bahia	-12.679969	-38.708236	Paiva et al., 2019
Ilha do Boi, Espírito Santo	-20.688281	-34.591222	Paiva et al., 2019
Gamboa, Espiríto Santo	-20.888769	-40.765536	Present study
Itaoca, Espírito Santo	-20.905027	-40.777093	Present study
Praia das Conchas, Rio de Janeiro	-22.871233	-41.980825	Paiva et al., 2019
Itaipu, Rio de Janeiro	-22.974086	-43.046942	Present study
Prainha, Paraty, Rio de Janeiro	-23.148219	-44.695563	Present study
Praia de Jabaquara, Paraty, Rio de Janeiro	-23.211277	-44.714141	Present study
Picinguaba, São Paulo	-23.357303	-44.865617	Paiva et al., 2019
Praia Dura, Ubatuba, São Paulo	-23.49265014	-45.1645267	Present study
Martin Sá, São Paulo	-23.625719	-45.375444	Paiva et al., 2019
Araçá, São Sebastião, São Paulo	-23.81756971	-45.40631776	Present study
Cananéia, São Paulo	-25.033333	-47.933333	Attolini et al., 1997
Ilha do Mel, Paraná	-25.5635	-48.318111	Ipucha et al., 2008

Table S6. The model's evaluation values for the all 10 replicates of four algorithms (Bioclim, Gower, SVM and Maxent). The TSS and AUC values were used to model evaluation. All values are more than 0,4 and 0,5, respectively. The TSS mean values and standard deviation (sd) are highlighted to each algorithm.

Table S7. *Perinereis ponteni* number of sequenced reads per specimen before and after filtering steps, in the final consensus and final number of loci. Abbreviations per specimen: Sabiaguaba (SBP); Prado Beach (PAP); Gamboa (GBP); Itaoca (ItaP); Prainha (PP); Jabaquara (JP); Dura Beach (DuP); Araçá (AP).

	# reads before filtering	# reads passed filters	# reads in cluster	# reads in clusters	# reads in consensus	# loci in assembly

				passed filter of depth		
AP1	11344063	11343640	98313	53806	50352	6042
AP10	4411843	4411691	72855	37606	35120	5964
AP2	4838433	4838240	69090	35760	33435	5961
AP3	3121747	3121622	62468	31506	29465	5744
AP4	6706177	6705912	80662	43249	40398	5979
AP5	5047669	5047511	71995	38834	36293	5934
AP6	5013925	5013738	71770	38241	35750	5933
AP7	7553081	7552705	88496	47415	44410	6020
AP8	5346715	5346465	77583	41111	38418	5970
AP9	3609083	3608896	67237	32725	30512	5854
DuP1	2493804	2493618	53355	18425	17187	4388
DuP10	8999232	8998686	76676	32606	30439	6032
DuP2	2682190	2682023	57028	20307	18924	4816
DuP3	5515777	5515466	67307	27872	25954	5827
DuP4	2375060	2374931	46426	17458	16197	4098
DuP5	1808710	1808615	44399	17407	16222	4241
DuP6	2249709	2249589	46469	16258	15152	3779
DuP7	4566768	4566538	60068	25023	23330	5556
DuP8	11117693	11117042	79460	36228	33804	6062
DuP9	13351968	13351002	97009	42036	39255	6087
GBP1	2020237	2020142	58771	24452	22827	5509
GBP10	2115461	2115357	63355	26345	24472	5526
GBP2	2798116	2797898	70550	30644	28485	5758
GBP3	3892751	3892583	71611	33500	31333	6003
GBP4	2911588	2911432	65266	26994	25200	5726
GBP5	5084282	5084070	75743	36605	34162	6047
GBP7	4956204	4955979	84927	35619	33172	6038

GBP8	4900938	4900703	76619	35775	33314	6039
GBP9	4122661	4122457	76269	33015	30776	5994
ItaP1	3478616	3478398	54259	20243	18834	4661
ItaP10	9378725	9378271	99999	42750	39926	6101
ItaP2	4042320	4042034	63360	27191	25332	5638
ItaP3	6497255	6496831	72633	31135	29065	5951
ItaP4	9028019	9027500	80498	33162	30935	6040
ItaP5	5166703	5166344	66684	26949	25165	5699
ItaP6	6422323	6421962	77298	30051	27958	5907
ItaP7	1669766	1669469	53355	17470	16395	3559
ItaP8	4192341	4192075	65967	26418	24609	5453
ItaP9	5395251	5394988	81536	35385	33018	6003
JP1	888534	888528	61564	26433	24764	5445
JP2	4718295	4718270	138974	62538	58615	6032
PAP1	2411668	2411649	92426	42023	39204	5977
PAP2	1743375	1743357	94582	38987	36319	5953
PAP3	607246	607233	50854	18455	17262	4317
PAP4	4298338	4298300	135603	59676	55716	5998
PAP5	5576002	5575957	144370	68726	64176	5999
PP2	4359988	4359970	135520	58304	54466	6019
PP3	4251463	4251447	141100	63096	59087	6005
PP4	5667036	5666987	148752	67478	63185	6018
PP5	7368488	7368457	173588	72679	68137	6043
PP6	3030788	3030772	110533	53393	50052	5910
PP7	3169027	3169005	114144	54198	50780	5986
SBP1	6188686	6188410	94361	47262	43995	5968
SBP10	7383054	7382802	86128	46202	43174	5991
SBP2	7391498	7391201	89715	47682	44584	5992
SBP3	2486116	2485965	64532	30650	28851	5361

SBP4	5329541	5329359	82321	41779	38993	5988
SBP5	3773103	3772821	87072	39793	37136	5871
SBP6	5638896	5638656	80254	43085	40156	5976
SBP7	2300639	2300554	62100	27930	26178	5280
SBP8	6161489	6161200	86285	44977	42316	5937

Table S8. *Perinereis ponteni* number of samples (n), observed heterozygosity (H_O), expected heterozygosity (H_E), and fixation coefficient (F_{IS}) values across all loci per sample. Geographic regions are given in brackets. *p value < 0.05; **p value < 0.01. Abbreviations are as in Table 1.

Location	**n**	**H_O**	**H_E**	**F_{IS}**
SBP (NE)	9	0.190	0.240	0.159**
PAP (NE)	5	0.243	0.325	0.172**
GBP (SE)	9	0.194	0.240	0.118**
ItaP (SE)	10	0.181	0.230	0.147**
PP (SE)	6	0.245	0.293	0.120**
JP (SE)	2	0.437	0.551	0.143
DuP (SE)	10	0.192	0.229	0.128**
AP (SE)	10	0.196	0.234	0.120**

Table S9. *Perinereis ponteni* pairwise F_{ST} (below diagonal) and p-values (above diagonal) between all sampling sites with all loci considered. Statistically significant values are in bold.

	AP (SE)	DuP (SE)	JP (SE)	PP (SE)	GBP (SE)	ItaP (SE)	PAP (NE)	SBP (NE)
AP (SE)	*	0.892	0.018	0	0	0	0	0
DuP (SE)	0.004	*	0.018	0	0	0	0	0
JP (SE)	**0.029**	**0.034**	*	0.054	0.009	0.009	0.036	0.009
PP (SE)	**0.026**	**0.027**	0.043	*	0	0	0	0
GBP (SE)	**0.037**	**0.035**	**0.060**	**0.034**	*	0.946	0.018	0
ItaP (SE)	**0.032**	**0.031**	**0.064**	**0.040**	0.003	*	0.009	0
PAP (NE)	**0.037**	**0.040**	**0.059**	**0.052**	**0.018**	**0.022**	*	0.496
SBP (NE)	**0.033**	**0.033**	**0.056**	**0.046**	**0.018**	**0.017**	0.013	*

Table S10. *Perinereis ponteni* AMOVA results and global fixation indexes with all loci considered.

Source of variation	**d.f.**	**Sum of squares**	**Variance components**	**Percentage of variation**	**Global fixation indexes**
Among groups	1	627.698	5	2.1	$F_{ST} = 0.037$
Among populations within groups	6	1,821.367	2	0.71	$F_{SC} = 0.017$
Among individuals within populations	53	14,708.394	33	13.14	$F_{IS} = 0.135$
Within individuals	61	12,895.500	211	84.05	-

Table S11. *Perinereis ponteni* isolation-by-environment partial Mantel environmental variables and results. Significant results in bold.

Recent		
Environmental variable	**Mantel statistic r**	**p-value**
Bathymetry	-0.121	0.672
Bathymetric slope	-0.099	0.677
Concavity	-0.034	0.533
Distance to shore	0.255	0.167
East/West aspect	-0.028	0.454
North/South Aspect	-0.143	0.762
Plan curvature	-0.204	1.000
Profile curvature	0.089	0.242
Sea surface salinity (annual mean)	-0.072	0.594
Sea surface salinity (monthly minimum)	-0.086	0.630
Sea surface salinity (monthly maximum)	-0.049	0.513
Sea surface salinity (annual range)	-0.072	0.632
Sea surface salinity (annual variance)	0.1066	0.779
Sea surface temperature (annual mean)	**0.409**	**0.022**
Sea surface temperature (coldest ice-free month)	0.189	0.135
Sea surface temperature (warmest ice-free month)	**0.368**	**0.041**
Sea surface temperature (range)	-0.095	0.610
Sea surface temperature (variance)	-0.092	0.614

Mid-Holocene		
Environmental variable	**Mantel statistic r**	**p-value**
Bathymetry	-0.121	0.651
Bathymetric slope	-0.099	0.702
Concavity	-0.034	0.558
Distance to shore	0.255	0.168
East/West aspect	-0.028	0.475
North/South Aspect	-0.143	0.740
Plan curvature	-0.204	1.000
Profile curvature	0.089	0.207
Sea surface salinity (annual mean)	-0.075	0.623
Sea surface salinity (monthly minimum)	-0.094	0.676
Sea surface salinity (monthly maximum)	-0.039	0.423
Sea surface salinity (annual range)	-0.035	0.498
Sea surface salinity (annual variance)	-0.080	0.727
Sea surface temperature (annual mean)	**0.413**	**0.027**
Sea surface temperature (coldest ice-free month)	**0.229**	**0.068**
Sea surface temperature (warmest ice-free month)	**0.369**	**0.047**
Sea surface temperature (range)	-0.1000	0.598
Sea surface temperature (variance)	-0.098	0.625

Table S12. Environmental variables selected for LFMM association. The number of SNPs associated with each variable is presented.

Source database	Variable #	Variable Name	# SNPs associated
Bio-Oracle	Variable 1	Chlorophyll A, maximum at the sea surface	304 SNPs
	Variable 2	Carbon phytoplankton biomass, mean at min depth	622 SNPs
	Variable 3	pH at the sea surface	600 SNPs
	Variable 4	Nitrate concentration, the range at min depth	256 SNPs
WorldClim	Variable 5	Temperature Seasonality (standard deviation ×100)	350 SNPs
	Variable 6	Minimum Temperature of Coldest Month	492 SNPs
	Variable 7	Mean Temperature of Wettest Quarter	329 SNPs
	Variable 8	Mean Temperature of Warmest Quarter	306 SNPs

Table S13. *Perinereis ponteni* Genbank hits of the outlier SNPs identified through Blast searches against nt and nr databases (minimum e-value e^{-10}).

Source of outlier SNP	Blast Hit	Species	Accession code	e-value
Chlorophyll A	Kinase heavy chain	*Platynereis dumerilii*	AUG84423.1	7.70E-14
Carbon phytoplankton biomass	Myosin-11-like	*Pteropus vampyrus*	XP_011385608.1	3.07E-13
Carbon phytoplankton biomass	Myosin-10-like	*Archocentrus centrarchus*	XP_030592138.1	3.70E-13
Carbon phytoplankton biomass	Helicase with zinc finger domain 2-like	*Anneissia japonica*	XP_033113853.1	1.77E-11
pH	Myosin-11-like	*Pteropus vampyrus*	XP_011385608.1	3.07E-13
pH	Kinase heavy chain	*Platynereis dumerilii*	AUG84423.1	7.70E-14
pH	Glycine dehydrogenase (decarboxylating)	*Pomacea canaliculata*	XP_025110614.1	1.39E-13
pH	Helicase with zinc finger domain	*Anneissia japonica*	XP_033113853.1	1.77E-11
Nitrate concentration	Kinase heavy chain	*Platynereis dumerilii*	AUG84423.1	7.70E-14

Min Temp Cold Month	Helicase with zinc finger domain 2	*Anneissia japonica*	XP_033113853.1	1.77E-11
Mean Temp Wet Quart	Kinase heavy chain	*Platynereis dumerilii*	AUG84423.1	7.70E-14

Table S14. Summary of all outlier SNPs per variable and software where each was identified for *Perinereis ponteni* .

Supplemental Figure Captions

Figure S1. Loading plot showing the contribution of each SNP allele in the PCA of *Perinereis ponteni*. Each horizontal bar represents one allele of each SNP. The cut-off was set as 0.46 to select the 100 SNPs with higher contribution to PCA structure.

Figure S2. Likelihood plot for each K value tested in Structure for *Perinereis ponteni*.

Figure S3. Hzar results for the best-fit model from the 16-model comparison in using the 14 neutral SNPs allele frequencies for *Perinereis ponteni.* In the plot of the maximum-likelihood cline, red dashed lines mark the medium slope cline.

Figure S4. BayeScan plot of 23,300 SNPs from 61 specimens of *Perinereis ponteni*. F_{ST} is plotted against the log10 of the False Discovery Rate (FDR). Vertical line shows the critical FDR used for outlier identification (FDR < 0.01).

Figure S5. Manhattan plots of LFMM results using the four Bio-Oracle variables described in table 4 and 23,300 SNPs from 61 specimens of *Perinereis ponteni.* Red dots represent outlier SNPs.

Figure S6. Manhattan plots of LFMM results using the four World Clim variables described in table 4 and 23,300 SNPs from *Perinereis ponteni* 61 specimens. Red dots represent outlier SNPs.

Figure S7. Redundancy analysis (RDA) results for genomic dataset of *Perinereis ponteni.* (a) The individual genotypes' association with each environmental predictor, where individuals from distinct locations are represented by different colors according to the legend, and (b) the projection of all SNPs significantly associated with each of the variables following the color code (p-value < 0.05).

Figure S8. Structure Harvester results indicating K = 3 as the most probable K value (right) and a bar chart showing Structure results for K = 3 (left) for the outlier SNPs. Each bar represents an individual of *Perinereis ponteni* and each color represents a different ancestry. Horizontal lines delimit the sampling sites.

Figure S9. Structure Harvester results indicating K = 2 as the most probable K value (right) and bar chart showing Structure results for K = 2 (left) for the 14,879 putatively neutral SNPs. Each bar represents an individual of *Perinereis ponteni* and each color represents a different ancestry. Horizontal lines delimit the sampling sites.