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ABSTRACT: Understanding the multiplicity of processes producing genetic patterns in natural populations can shed light on the ecology and evolution of species, and help guide effective management and conservation strategies. Here we investigated the role of environmental, demographic, and geographic factors in shaping the spatial patterns of genetic diversity and differentiation of the intertidal barnacle *Notochthamalus scabrosus* along the central-northern coast of Chile (28–34° S). We analyzed genetic data from 7 microsatellite loci genotyped for 300 individuals sampled from 10 sites and combined this

information with 8 site-specific environmental (4), demographic (2), and geographic (2) variables using least squares linear regressions, generalized linear models, and matrix regression analyses. We found a strong association between the spatially structured genetic diversity of *N. scabrosus* and patterns of temporal variability in chlorophyll *a*, and among-site differences in seawater temperature and adult abundance, which in turn was related to overall recruitment levels. Our results illustrate that population size, partly driven by recruitment success, can leave a signal on genetic structure of this highly dispersive marine species. The significant effect of temperature and chlorophyll *a* stresses that local adaptation may be key to understanding the spatial genetic structure of our model species. Hence, the results of this work represent an advance towards understanding the usually complex causal relationships between environmental variables, gene flow, and genetic diversity patterns of coastal populations.

KEY WORDS: *Notochthamalus scabrosus* · Seascape genetics · Larval dispersal · Coastal oceanography · Marine connectivity

1. INTRODUCTION

Population genetic diversity is important for a range of ecological and evolutionary processes. For example, genetic diversity can determine community structure and primary productivity (Crutsinger et al. 2006). It can be associated with the population growth rate of species (Hughes et al. 2008), and it allows species to adapt to changing environments and fosters persistence over evolutionary time scales (Reed & Frankham 2003). Therefore, a strong scientific understanding of the processes that influence spatial genetic variation, genetic diversity, and population structure in nature is of paramount importance to implement efficient conservation and management strategies.

In marine systems, an early paradigm assumed that most organisms were highly dispersive and presented large population sizes, and thus were able to resist genetic divergence at all but perhaps the largest spatial scales. This overly simplistic view was gradually replaced by an increased understanding of hydrographic heterogeneity in the coastal ocean and the advent of molecular genetics, which unveiled many potential causes for genetic structure and speciation in organisms with large dispersal potential (Hellberg 2009, Selkoe et al. 2016). For example, due to the dependence of genetic diversity on population effective size (Ellegren & Galtier 2016), demographic changes along the geographic range of a species can leave a discernible footprint in its spatial genetic makeup. Also, a realistic oceanic environment, especially when there is strong topographic modulation, presents ample opportunities for variation and restriction in effective dispersal distances (Largier 2003, Pringle et al. 2011, Nickols et al. 2015). Geographic distance per se imposes a distance limitation to gene flow, driving increased genetic differentiation with increasing distance between populations, a pattern known as isolation by distance (Wright 1943). Besides the limitation imposed by dispersal between distant populations, phenotype–environment mismatches can impose biological barriers to gene flow (Nosil et al. 2005), producing an isolation by environment, where populations with greater environmental dissimilarity exhibit higher levels of genetic differentiation, blurring or reinforcing patterns generated by geographic distance alone (Wang & Bradburg 2014).

High-resolution molecular and environmental data are now routinely used to assess the influence of landscape-scale environmental characteristics on genetic variation and

spatial patterns in natural populations of species. Altogether, the mounting evidence
suggests that considerable genetic structure occurs in marine populations around areas
where environmental oceanographic factors exhibit strong spatial structure or geographic
discontinuity.

The central-northern coast of Chile represents an interesting study system to
evaluate the effects of environmental, demographic and geographic factors on genetic
diversity and differentiation of marine organisms. Superimposed on what are smooth
latitudinal trends in mean sea surface temperature (SST) along this highly productive
upwelling ecosystem, there is a marked change in oceanographic regimes that takes place
around 30° S. Such geographic discontinuity entails changes in upwelling-driven coastal
circulation (Hormazabal et al. 2004, Aiken et al. 2011, Aguirre et al. 2014), as well as
prevailing hydrographic conditions, such as SST variability, surface chlorophyll, and
nutrient availability (Navarrete et al. 2005, Tapia et al. 2014). Coincidentally, at this same
latitude, several studies have reported the occurrence of geographic distribution endpoints
of several intertidal and subtidal invertebrate species (Lancellotti & Vasquez 1999, Camus
2001), phylogeographic breaks of several invertebrates and macroalgae (Tellier et al. 2009,
Haye et al. 2014), and large changes in population dynamics and abundance of dominant
rocky shore species that otherwise extend far beyond this region (Broitman et al. 2001,
Navarrete et al. 2005, 2008).

The geographic range of the intertidal barnacle *Notochthamalus scabrosus* (Darwin
1854) spans the 30° S transition zone, and its complete larval development to settlement
takes well over 1 mo at the water temperatures typically encountered in central Chile
(Venegas et al. 2000). At the same time, the advective nature of coastal flow along central
Chile (Aiken et al. 2007) sets the stage for a comparatively high potential for larval
dispersal and genetic flow among distant populations of this species, as shown by
biophysical models for other long-distance dispersers in the region (Garavelli et al. 2014).
Moreover, large variation in larval arrival rates and adult cover have been reported for the
central-northern coast, which has been attributed to differences in the temporal regime of
upwelling-favorable winds (Navarrete et al. 2005, Lagos et al. 2008). A phylogenetic break
in the *N. scabrosus* mitochondrial cytochrome oxidase I gene (mtCOI) around 30° S was
reported by Zakas et al. (2009). Although spatially stable, there are significant temporal
changes in gene frequencies near the break (< 30° 55' S), presumably related to source–sink
dynamics and/or low effective population sizes in this zone (Laughlin et al. 2012). Based
on a large-scale circulation model, Ewers-Saucedo et al. (2016) suggested that the genetic
break of *N. scabrosus* around 30° S requires differential performance of mtCOI lineages
along the coast; in other words, it could not be maintained by dispersal limitation alone.
Therefore, the diversity and genetic structure of *N. scabrosus* may respond to multiple
causes, such as phylogeography, demography, geographical isolation, and selection
pressures driven by environmental variation along the coastline.

This study takes advantage of the genetic information gathered for *N. scabrosus*,
based on mtCOI, as well as of a long-term database (5–13 yr) of monthly larval arrival
(recruitment) of this species at multiple sites spanning the reported latitudinal break.
Together with surveys of adult abundance and satellite-based information of environmental
(oceanographic) variables for the region (28–34° S), and the development of neutral
microsatellite markers, we assessed the potential influence of nearshore environmental,

demographic, and geographic factors on the genetic diversity and population structure patterns of *N. scabrosus*. Using neutral markers of gene flow allowed us to (1) characterize patterns of genetic diversity and the spatial genetic structure in *N. scabrosus* and (2) determine the relative importance of environmental, demographic, and geographic factors for genetic variation between and within populations of this widely distributed barnacle species.

2. MATERIALS AND METHODS

2.1. Hydrography of the study area

The coast of Chile between 18 and 42° S is under the broad influence of the northward flowing Humboldt Current (also called Chile–Peru Current). Close to shore, coastal hydrography is dominated by the dynamics of the Chilean Coastal Current (CCC), a predominantly northward surface stream forced by the prevailing south and southwest upwelling-favorable winds (Aiken et al. 2008, 2011), which intensify during spring and early summer months, and around coastal topographic features (Strub et al. 1998, Tapia et al. 2009, Bravo et al. 2016). Our study area is located in a fairly straight shoreline stretch (Fig. 1) and is exposed to direct wave action (Narváez et al. 2006).

Within the study area, the main upwelling centers are Punta Talca, Punta Toro, Curaumilla, Pichilemu, and, to a lesser extent, Los Molles (Silva & Valdenegro 2003, Wieters et al. 2003, Tapia et al. 2009, 2014). In contrast, the bays of Cartagena, Valparaíso, and Coquimbo remain relatively protected from upwelling (Kaplan et al. 2003, Vargas et al. 2004, Aiken et al. 2008). Four sampling sites (PTal, LMol, Cura, and Pich) were located in active upwelling centers, and 4 sites, namely Temb and Guan (Coquimbo Bay), Mont (Valparaíso Bay), and ECIM (Cartagena Bay), were located in places of weak upwelling. For the 2 remaining sites (Apol and CBaj), records from *in situ* SST suggest that the hydrography of Apol may be similar to that of weak upwelling sites, while CBaj seems to be under the influence of active upwelling (Valdivia et al. 2015).

An important geographic discontinuity in upwelling-favorable winds occurs around 30–32° S (Strub et al. 1998, Thomas 1999, Hormazabal et al. 2004, Navarrete et al. 2005). North of this latitude, equatorward winds are weaker but more persistent throughout the year, while to the south winds are stronger but temporarily more variable (Hormazabal et al. 2004, Navarrete et al. 2005). The change in oceanographic regimes determines or modulates the concentration and temporal variability of surface phytoplankton (Thomas 1999), nutrient regimes (NO_3) of coastal waters (Tapia et al. 2014), and functional structure of benthic communities (Broitman et al. 2001, Navarrete et al. 2005, Wieters et al. 2009).

2.2. Study species

Notochthamalus scabrosus is distributed along most of the rocky coasts of Ecuador, Peru, and Chile (Brattström & Johanssen 1983). In the zone occupied by chthamalid barnacles, *N. scabrosus* inhabits the 3 intertidal elevations, with greater abundance in the middle and upper intertidal zones (Paine et al. 1985, Shinen & Navarrete 2010). Adults are sessile filter feeding, hermaphroditic brooders. The life cycle of *N. scabrosus* includes a pelagic larval stage that lasts about 37 d at 15–18°C, with 6 naupliar stages with planktotrophic feeding and a cyprid stage competent for settlement (Venegas et al. 2000).

169 Cyprid settlement occurs in pulses of larval arrival to the coast during a few days within the
170 recruitment period, which is mainly concentrated in spring–summer (Tapia & Navarrete
171 2010).

2.3. Sampling of individuals, DNA extraction, and genotyping of microsatellites

173 At each of 10 study sites, 30 *N. scabrosus* adults of 3–6.4 mm rostrocranial length
174 were collected from rocky platforms approximately 10–30 m long × 4–8 m wide.
175 Individual barnacles were identified as *N. scabrosus* in the field and were removed from the
176 rock with a scalpel and immediately stored in tubes with 95% ethanol for preservation.
177 Total DNA was extracted using the salt/Proteinase K method (Aljanabi & Martinez 1997)
178 and quantified in a spectrophotometer (Nanodrop).

179 Seven microsatellite loci were amplified by polymerase chain reaction (PCR). The
180 microsatellite development procedure, the conditions under which the PCRs were
181 performed, and the GenBank accession numbers can be found in Table S1 in the
182 Supplement at www.int-res/articles/suppl/m123p456_supp.pdf. Alleles were identified by
183 capillary electrophoresis in an ABI3130 Genetic Analyzer (Applied Biosystems), and the
184 Excel FLEXIBIN macro (Amos et al. 2007) was used to calibrate the reading and allele
185 binning of each locus.

2.4. Genetic polymorphism

187 The total number of observed alleles (Na), number of private alleles (Pa), and
188 observed (H_o) and expected (H_e) heterozygosity were calculated in GENALEX 6.5 (Peakall
189 & Smouse 2012). Per locus gene diversity (Gd) and standardized allelic richness (Ar) were
190 calculated in the FSTAT software version 2.9.3.2 (Goudet 2001). The Ar index was
191 calculated using the rarefaction method to avoid bias due to differences in sample size
192 (Leberg 2002). To evaluate deviations from the Hardy-Weinberg expectations (HWE),
193 Fisher's exact tests were performed for heterozygote deficits at each site–locus
194 combination, and *U*-score tests for global HWE per site through loci and per locus across
195 sites (dememorization 10000; 100 batches; 10000 iterations) using the GENEPOP 4.2
196 software (Rousset 2008). Linkage disequilibrium between all pairs of loci at each site and
197 between each pair of loci across sites was assessed by Fisher's exact tests implemented in
198 GENEPOP with this same parameter set. The inbreeding coefficient F_{IS} by locus and site
199 was quantified with GENETIX 4.05 (Belkhir et al. 2004), and departures from random
200 expectations were assessed by 10000 permutations. For all multiple comparisons, the
201 nominal level of significance of 5% was adjusted using the false discovery rate (FDR;
202 Benjamini & Hochberg 1995).

203 To test for large allele dropout and stuttering and to estimate the frequency of null
204 alleles at each site–locus combination following Brookfield (1996: Eq. 4), data were
205 analyzed with the MICROCHECKER software (van Oosterhout et al. 2004).

2.5. Population genetic structure

207 Global and pairwise genetic differentiation was evaluated calculating θ_{ST} (Weir &
208 Cockerham 1984) and D_{EST} (Jost 2008) indices, in GENALEX 6.5 (Peakall & Smouse
209 2012) and running 10000 permutations to evaluate their significance. Jost's D_{EST}
210 outperforms G_{ST} and its relatives (F_{ST}) over a range of sample sizes, including in situations

where we have highly variable microsatellite loci with different numbers of alleles (Heller & Siegismund 2009, Gerlach et al. 2010), but it is recommended to compare results between differentiation indices (Leng & Zhang 2011). In all multiple comparisons, sites were used as population units, and the nominal level of significance, 5%, was adjusted using FDR. To identify population relationships in a 2-dimensional space, principal coordinate analyses (PCoAs) of the sites were computed and graphed in GENALEX 6.5 using the θ_{ST} and D_{EST} differentiation indices.

As null alleles can impose error in differentiation estimates (Pompanon et al. 2005), 2 approximations were conducted. First, using MICROCHECKER, we obtained a new database corrected for null alleles. MICROCHECKER adjusts the number of homozygote genotypes to reflect the estimated frequency of null alleles and the likely number of homozygotes given the adjusted allele frequencies and assuming random mating. We then repeated the previous differentiation analysis using the database adjusted by the frequency of null alleles. Second, pairwise F_{ST} with and without the null allele correction was estimated using the expectation-maximization (EM) algorithm (Dempster et al. 1977) with ENA correction to give an accurate estimate of F_{ST} in the presence of null alleles using FREENA (Chapuis & Estoup 2007). The uncorrected and corrected pairwise F_{ST} were then compared by means of a paired t-test.

To estimate the number of genetically differentiated groups, Bayesian-based clustering was used as implemented in STRUCTURE 2.3.4 (Pritchard et al. 2000). STRUCTURE was run using the admixture model, the assumption of correlated allelic frequencies between clusters, with and without the recessive alleles option that accounts for the null alleles (Falush et al. 2003, 2007), and considering sampling site information (Hubisz et al. 2009). From Zakas et al. (2009) and Laughlin et al. (2012), we know that $k = 1$ can be rejected, so all runs were made for k values between 2 and 10. Ten independent runs with 500000 Markov chain Monte Carlo replicates and a burn-in length of 50000 were used for each value of k. In order to select the k value that best captures the structure of the data, the statistic Δk, a measure of the second-order rate of change in the likelihood of k (Evanno et al. 2005) was implemented in STRUCTURE HARVESTER (Earl & vonHoldt 2012), and the values of Δk as a function of k were plotted. In CLUMPP 1.1 (Jakobsson & Rosenberg 2007), we merged the results of the 10 runs for each value of k, and DISTRUCT 1.1 (Rosenberg 2004) was used to graphically visualize the results.

2.6. Demographic variables

2.6.1. Recruitment rates

At each site, an estimate of arrival rates of larval *N. scabrosus* was obtained by quantifying recruitment onto 10×10 cm Plexiglas plates covered with SafetyWalk^{TM} (3M), an anti-slip surface that provides a heterogeneous substrate for larvae settlement and ensures homogeneity of conditions across plates and sites (Menge 2000). Five replicate collectors were fastened to the rocky substrate with stainless-steel bolts in the mid-upper intertidal zones of rocky platforms exposed to swell. Replicate collectors were replaced monthly, and recruitment rates were standardized to the number of ind. collector^{-1} d^{-1}. The monthly recruitment rates were then averaged to obtain the annual recruitment rates, and these in turn were averaged over the years to estimate the per site recruitment rate. At 8 of the 10 study sites, the collectors were initially deployed in late 1999 or early 2000, whereas

at the 2 northernmost sites (CBaj and Apol), recruitment surveys began in mid-2009. The recruitment time series used here covered the period up to December 2013 for all sites.

2.6.2. Adult cover (abundance)

At each site, the benthic abundance of *N. scabrosus* was estimated using 7 to 10 quadrats of 0.25 m^2, located along ca. 20–30 m alongshore transects. Transects were repeated at 3 intertidal elevations (low, mid-, and high intertidal zones) of the same rocky platforms where we deployed larval collectors. The 50×50 cm quadrat frame was divided into 25 equal squares with monofilament line, which was used to visually estimate adult abundance of *N. scabrosus* as percentage cover. Cover surveys were conducted approximately every 6 mo. For more details about the field methods, see Broitman et al. (2011).

2.7. Environmental and geographic variables

Environmental heterogeneity imposed by hydrographic conditions such as SST and productivity can directly or indirectly affect population genetic structure in marine organisms (Bekkevold et al. 2005, Mendez et al. 2010, Wei et al. 2013). A multivariate indicator of environmental variability was constructed to test for correlation with the spatial genetic structure of *N. scabrosus*. To this end, spatio-temporal variations in chlorophyll *a* (chl *a*) concentration (mg m^{-3}) and SST (°C) over a period of 10 yr (January 2003 to December 2013) were processed from monthly averages of Aqua MODIS satellite data with a 4 km spatial resolution using MatLab R2014a. Temporal variability in chl *a* and SST across the region was then decomposed by 2 separate principal component analyses (PCAs) of the respective time series, so that scores of sites on PC1 and PC2 (typically called empirical orthogonal function (EOF1 and EOF2) in the oceanographic literature, as they are carried out in the time domain), were used as multivariate representations of environmental conditions for either SST or chl *a*. In these analyses, the first axis (PC1) is dominated by the seasonal amplitude, with positive/negative values corresponding to sites with strong/weak seasonality. The second mode (PC2) is dominated by higher frequency variability, which in our system is chiefly synoptic variation corresponding to upwelling dynamics (see Wieters et al. 2009, Tapia et al. 2014, Valdivia et al. 2015 for similar analyses). Latitudinal (Lat) and longitudinal (Lon) positions of each site were used as descriptors of geographic structure.

2.8. Environmental/demographic/geographic–genetic association analysis

Three complementary approaches, i.e. simple linear regressions, multi model selection, and matrix regression, were employed to test for associations among environmental, demographic, and geographic factors with the spatially structured genetic diversity of *N. scabrosus*. The linear regressions and model selection analyses used location (site)-specific data to evaluate the influence of our explicative variables on genetic diversity across sampling sites. The matrix regression analyses used the explicative variables as a proxy of 'seascape resistance' (McRae 2006) to evaluate their effects on among-site genetic differentiation. Our 8 predictive variables were the PC1 and PC2 of chl *a* and SST (hereafter CHLA1, CHLA2, SST1, and SST2), long-term averages of recruitment rates (Rec) and adult cover (Cov), and geographic location (Lat and Lon). The variables Rec and Cov were log$_{10}$ transformed before analyses to approximate normal distributions.

First, we conducted least squares (LS) linear multiple regression analyses between each metric of genetic diversity (Ar and Gd) and our 8 predictive variables. Second, a sensitivity analysis was performed using generalized linear models (GLMs) to select the best model of variables to explain spatial variation in our metrics of genetic diversity. Because some predictor variables are highly correlated (see Table S3), we examined the impact of collinearity using the variance inflation factor (VIF) before running analyses. The variables Lat, Rec, and SST2 showed high (>10) VIF values, and were removed to minimize VIF values (<5). We then followed a stepwise approach for the sensitivity analysis, which was repeated for the 2 dependent variables (Ar and Gd) in R (R Core Development Team 2017): We (1) ran a full GLM that includes all predictive variables with VIF values <5; (2) examined the scatterplot of residuals versus predicted values (both in terms of the slope of the relationship and in the dispersion of the values) to check for the absence of trends; (3) sequentially removed (one by one) all predictive variables that were not significant ($p > 0.05$); (4) selected the most parsimonious 'suboptimal' GLM through a manual stepwise procedure according to the relative contribution of each factor to the variance explained by the model retaining predictive variables with a relative contribution $\geq 10\%$; (5) computed LS means of the dependent variable for each model parameter in order to assess the effect of specific variables on the dependent variables.

As an alternative way to obtain the best subset of explicative variables, we performed stepwise selection (both forward and backward) using the stepAIC function from the 'mass' package in R. This function uses the exact Akaike's information criterion (AIC) as the model selection criterion. Third, we used multiple regressions on distance matrices (MRDM; Manly 1986, Legendre et al. 1994) to estimate the independent effects of explicative variables on *N. scabrosus* among-site genetic differentiation. Briefly, partial regression slopes were estimated using standard multiple linear regression, but the significance of each term was determined by randomly permuting the explanatory variables one at a time while keeping the others constant (Wang 2013). This analysis was implemented using the 'ecodist' package (Goslee & Urban 2007) in R, and significance was based on 10000 permutations using the genetic distance matrices (θ_{ST} and D_{EST}) as response variables. Each of 8 matrices representing environmental, demographic, and geographic differences among sites were used as the predictor variables. Data were converted into matrices of pairwise distances calculating the absolute differences from site-specific values of each variable following Amaral et al. (2012). Due to its high VIF value (>10), the geographical variable Lat was removed, so a subset of 7 predictor variables was retained for the subsequent analysis.

3. RESULTS

3.1. Genetic polymorphism

The 7 microsatellite loci reached a total of 158 alleles in the 300 individuals of *Notochthamalus scabrosus* genotyped, which ranged from 79 in CBaj to 95 in Temb. In addition, we found 27 private alleles, with the highest number observed at Apol (Pa = 6). In contrast, Pich shared all of its alleles with most other sites. The Ar ranged from 11.0 in CBaj to 13.2 in Temb (mean Ar = 12.01 ± 0.72), while Gd ranged from 0.77 in ECIM to 0.83 in Apol (mean Gd = 0.81 ± 0.02). Both Ar and Gd indices showed a peak at Guan,

341 Temb and Apol (located around Coquimbo Bay), while the lowest values were found at
342 CBaj and ECIM for Ar, and at ECIM and Mont for Gd (Table S2).

343   All 10 populations exhibited significant heterozygote deficiency. Of the 70 site–
344 locus combinations, 58 showed a significant deviation from HWE based on Fisher's exact
345 test and after FDR correction, while only 37 had significantly positive F_{IS}-values based on
346 a permutation test (Table S2). Using the corrected database for null alleles, 56 site–locus
347 combinations remained significantly deviating from HWE with the exact test, and 30 site–
348 locus comparisons still had significant $F_{IS} > 0$ with permutation tests (Table S2).

349   Of the 210 linkage disequilibrium tests performed, none was significant after
350 correcting for false positives (FDR), and none of the global tests for each pair of loci across
351 sites was significant. The estimated frequency of null alleles by site–locus combination
352 varied between 0 and 0.379, with an average frequency of 0.156 (SD = 0.089) across loci
353 and sites (Table S2).

3.2. Population genetic structure

355   *N. scabrosus* showed statistically significant global genetic structure ($\theta_{ST} = 0.013$, p
356 < 0.001; $D_{EST} = 0.040$, p < 0.001). Pairwise θ_{ST} and D_{EST} were significant for 27 and 21 of
357 the 45 comparisons at the nominal level ($\alpha = 0.05$), of which 22 and 15 remained
358 significant after corrections for multiple tests, respectively (Table 1). Significant pairwise
359 comparisons were mostly between sites north of PTal versus sites south of LMol, and the
360 sites with lowest and highest levels of differentiation were Guan vs. PTal (separated by
361 50.26 km) and Temb vs. Cura (separated by 419.88 km), respectively. CBaj had the highest
362 number of significant pairwise comparisons for both θ_{ST} and D_{EST} (n = 9 and 8,
363 respectively) followed by LMol (n = 7 and 6, respectively; Table 1). The first 2 coordinates
364 of PCoAs with θ_{ST} and D_{EST} values explained 87.62 and 87.88% of total variation,
365 respectively, and revealed similar structuring of sites (Fig. 2). The first axis of the PCoAs
366 separated 2 principal groups, one composed of sites from LMol to the south, the other with
367 the 3 northern sites (Temb, Apol, and CBaj), whilst Guan and PTal were between these 2
368 groups. Weak separation of sites within these regions was detected along the second PCoA
369 axis, with Temb separated from Apol and CBaj, and LMol and Cura from Mont, ECIM,
370 and Pich (Fig. 2).

371   Null alleles had some effect on our results: (1) the ENA method gave slightly, but
372 significantly, lower F_{ST} values (average F_{ST} with ENA = 0.00815, SD = 0.00693) than
373 those obtained without correction for the presence of null alleles (average F_{ST} without ENA
374 = 0.00951, SD = 0.00847; paired $t = 3.74$, p < 0.001); (2) global structure was lower but
375 still significant with the adjusted database ($\theta_{ST} = 0.012$, p < 0.001; $D_{EST} = 0.018$, p =
376 0.001); and (3) there were fewer significant pairwise comparisons after FDR corrections for
377 D_{EST} (only 1 significant comparison) and θ_{ST} (15 of 22 comparisons still significant).
378 However, the main pattern of differentiation between sites north and south of PTal–LMol
379 persisted with the adjusted database, as well as the most and least differentiated pairwise
380 comparisons (Table 1).

381   The cluster analysis performed using STRUCTURE confirmed the existence of 2
382 clusters, one south of LMol and the other north of PTal (Fig. 3). For $k = 3$, a new cluster
383 included CBaj, the northernmost site. According to Evanno's criteria, $k = 4$ was the most
384 likely number of clusters (Fig. S1). However, no clear spatial pattern could be recovered

385 from the assignment of individuals into these 4 clusters. This may be due to the correlated
386 allele frequencies model, which tolerates differentiation of closely related populations, but
387 is likely to overestimate k (Pritchard et al. 2000). The same trends were observed with the
388 full or the adjusted databases (results not shown).

3.3. Demographic/geographic/environmental–genetic association analysis

390   Linear regressions showed that CHLA2 alone explained 48 and 61% of the total
391 variance in Ar and Gd, respectively, having a significant positive linear relationship with
392 both genetic diversity indices throughout the study region (Fig. 4). Additionally, Cov
393 explained 33% of the variance of Ar, and SST2 and Lat explained 25 and 30% of the
394 variance of Gd, respectively, but these relationships were not statistically significant (Fig.
395 4).

396   Statistical control of covariables using GLM model selection identified the variable
397 CHLA2 as the most significant factor explaining variation in both Ar and Gd (Table 2). The
398 second and third best models include the variables CHLA1 and Cov, which is consistent
399 with results of the model selection based on AIC (Table 3), but the fraction of variance
400 explained by these variables was minor in comparison to CHLA2 (see Table 2).

401   A different result was obtained from the MRDM analysis, which showed that the
402 spatial structure (differences among sites) in SST2 and Cov had the strongest effects on
403 genetic differentiation, as measured by θ_{ST} and D_{EST}. The overall model showed significant
404 fit to the data ($p < 0.05$), and explained 55% of the total variance (Table 4).

4. DISCUSSION

406   The extent of effective dispersal and gene flow between populations in the coastal
407 ocean can be much more complex than previously thought (e.g. Pringle & Wares 2007,
408 Teske et al. 2016). In the present study, we found subtle, yet significant levels of genetic
409 differentiation in the intertidal barnacle *Notochthamalus scabrosus*, a species with high
410 dispersal potential. Main differences occurred between sites located to the north and south
411 of the reported phylogeographic latitudinal break at 30° S.

412   Our results suggest that population genetic diversity in *N. scabrosus* is influenced
413 by environmental regimes manifested in patterns of temporal variability of surface chl *a*
414 concentration, whereas among-site differences in SST fluctuations and benthic abundance
415 of adults appear to be significant drivers of population genetic differentiation over space.
416 More broadly, the presence of sites that are both environmentally and genetically
417 differentiated supports the idea of an ecological restriction to population connectivity,
418 despite the long residence of larvae in the water column.

419   Larval arrival from the plankton can be responsible for local abundance and genetic
420 variability patterns of benthic populations (Iacchei et al. 2013). We found that the temporal
421 variability in surface chl *a* was the most consistent covariable explaining spatial distribution
422 of *N. scabrosus* genetic diversity. This general result is in line with studies showing that
423 patterns of intraspecific genetic diversity of some mobile marine species are associated with
424 variation in chlorophyll concentration (Gaggiotti et al. 2009, Mendez et al. 2010, Amaral et
425 al. 2012). Variability in coastal chl *a* may be viewed as an integrated indicator of the
426 environmental conditions to which invertebrate larvae and onshore adults are exposed, and

427   likely determines both the feeding conditions (i.e. quantity and quality of food) and the
428   larval transport to/off the shore. During upwelling, high food availability can translate into
429   better larval condition and, at the same time, offshore and alongshore upwelling currents
430   can promote the mixing of the offshore larval pool (Barshis et al. 2011). Then, during
431   upwelling relaxation and downwelling events, this well fed/well mixed larval pool can
432   reach local populations. In this manner, sites with constant strong upwelling have few
433   possibilities of larval arrival due to increased larval waste (Roughgarden et al. 1988, Menge
434   & Menge 2013), while on the other hand, sites with constant weak upwelling have more
435   larval retention, therefore their recruitment comes from a poorly mixed larval pool. Other
436   things being equal or homogeneous, high phytoplankton availability in coastal waters
437   during larval development can therefore lead to higher recruitment (e.g. Olson & Olson
438   1989, Cushing 1990, Menge 2000) and high larval physiological quality that should
439   improve post-settlement survival (Jarrett & Pechenik 1997, Hentschel & Emlet 2000,
440   Phillips 2002) as well as overall juvenile condition (Bertness et al. 1991, Menge et al. 1997,
441   Sanford & Menge 2001). All of these factors may result in the maintenance of genetic
442   diversity from the larval pool. Thus, variable upwelling will maximize larval condition and
443   genetic diversity and, as predicted by the intermittent upwelling hypothesis (Menge &
444   Menge 2013), increase onshore recruitment. Further genetic studies should therefore
445   intensify sampling of recently settled larvae across more diverse upwelling conditions.
446   Indirect evidence about the effect of upwelling/relaxation dynamics on barnacle recruitment
447   (Navarrete et al. 2005, Lagos et al. 2008) and the significant positive cross-correlations
448   between mean chl *a* concentration and *N. scabrosus* recruitment and adult abundance
449   (Table S3) suggest that it is a possible mechanism to explain the genetic pattern in *N.*
450   *scabrosus*.

451   Adult cover was used as a proxy of local abundance of *N. scabrosus*, a factor that in
452   linear regressions explained 33% of total variance in allelic richness (although it was not
453   statistically significant, Fig. 4). From examination of Fig. 4f, it seems clear that the site
454   ECIM deviates largely from an otherwise good positive relationship formed by the other 9
455   sites. Indeed, removing ECIM from the analysis increases the relationship to $r^2 = 0.57$ (p =
456   0.019). The departure of ECIM from the general pattern illustrates well the complexity of
457   determinants of genetic diversity in natural systems and why such univariate relationships
458   between population size and genetic diversity are rarely found in marine environments (but
459   see McCusker & Bentzen 2010). ECIM has some of the historically highest recruitment
460   rates for *N. scabrosus* in the region (Navarrete et al. 2008), yet it displays one of the lowest
461   levels of genetic diversity (in both Ar and Gd indices, Table S2). Furthermore, only at
462   ECIM did individuals have levels of relatedness significantly larger than expected from
463   HWE (Fig. S2).

464   ECIM is located within Cartagena Bay, an open bay exposed to the southern swell,
465   but in an 'upwelling shadow' where upwelling advection is largely reduced, apparently
466   leading to high phytoplankton concentration (Wieters et al. 2003) and stronger stratification
467   than other sites (Kaplan et al. 2003, Bonicelli et al. 2014). On other shores of the world,
468   low current velocities and water re-circulation, leading to increased local larval retention
469   (McShane et al. 1988), create distinctive patterns of genetic diversity in local populations
470   (e.g. Dupont et al. 2007, Nicastro et al. 2008, Olivares-Bañuelos et al. 2008). Thus,
471   increased larval retention at ECIM, with comparatively low immigration from other
472   populations, as suggested by numerical circulation models (Aiken et al. 2007, Ospina-

473 Alvarez et al. 2018) and observational studies (Bonicelli et al. 2014), may explain the
474 higher genetic relatedness levels observed at this site. The reduced gains of genetic
475 diversity from other sites (poorly mixed larval pool) may be the cause of reduced allelic
476 richness, further supporting the relevance of connectivity patterns on adult population size
477 and genetic diversity.

478   In natural populations, a genetic discontinuity along a continuously colonized range
479 can arise as a consequence of an environmental discontinuity, either through selection
480 against migrants or reduced fitness of interlineage hybrids (Nosil et al. 2005). Both
481 mechanisms involve local adaptation in response to selection imposed by divergent biotic
482 or abiotic conditions (Sanford & Kelly 2011, Pflüger & Balkenhol 2014). Our results
483 support the idea that 'environmental distance,' imposed by among-site differences in SST,
484 is a relevant factor to explain genetic differentiation among *N. scabrosus* populations.
485 Indeed, a similar effect has been observed in mammals (Fullard et al. 2000, Amaral et al.
486 2012), fishes (Han et al. 2012, Diopere et al. 2018), and intertidal and shallow (<5 m depth)
487 coastal invertebrate species (Banks et al. 2010, Wei et al. 2013). Seawater temperature is
488 also one of the most important factors controlling reproduction, development, and growth
489 of ectothermic invertebrates (Pechenik 1987, O'Connor et al. 2007, Byrne 2011). In the
490 case of *N. scabrosus*, such adaptive divergence could be related to selective sorting of
491 competent larvae and/or to post-settlement processes such as temperature requirements for
492 metamorphosis and initial growth, or desiccation tolerance of recruits. Further studies
493 combining genomic tools with high-resolution dispersal models and local experiments with
494 settlers are necessary to discern among the possible mechanisms of population divergence.

495   **5. CONCLUSION**

496   Population genetic structure of *Notochthamalus scabrosus*, as assessed by neutral
497 markers, is characterized by a sharp genetic discontinuity around 30° S, confirming
498 previous conclusions based on mtCOI (Zakas et al. 2009, Laughlin et al. 2012). A modeling
499 study by Ewers-Saucedo et al. (2016) showed that dispersal alone could not generate such
500 genetic discontinuity, and that differential lineage performance in adjacent but divergent
501 environments must be considered. Our results strongly suggest that the environment is
502 indeed influencing the spatial pattern of genetic diversity in *N. scabrosus*. Two main
503 mechanisms could be hypothesized: temporal variability of the food (variation in
504 phytoplankton abundance) and dispersive (upwelling-associated currents) coastal
505 environments favor recruitment from a well fed/well mixed larval pool and therefore
506 increase the allelic richness of benthic populations; and the ecological divergence in coastal
507 ocean temperature may restrict effective dispersal across the 30° S boundary. Such patterns
508 have not been observed in other barnacles, which are traditionally assumed to have large
509 effective population sizes and large dispersal capacity, both of which could override the
510 effects mentioned above. We interpret these results as suggestive that coastal circulation
511 can limit larval connectivity among some populations, generating incomplete barriers to
512 dispersal, which in turns facilitates effects of isolation by environment. Hence, the results
513 of this work advance our understanding of how environmental seascapes can shape patterns
514 of genetic diversity and population differentiation. In particular, our results highlight the
515 importance of further defining the causal relationships between environmental variables
516 and genetic diversity patterns of wild populations in order to guide future region-wide
517 conservation and management efforts.

527

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Table 1. Among-site genetic differentiation of *Notochthamalus scabrosus* at 7 microsatellite loci. Analyses were done with (A) the original database and (B) the database corrected for null alleles. Values of θ_{ST} are above the diagonal and D_{EST} values are below the diagonal. CBaj: Carrizal Bajo, Apol: Apolillado, Temb: Temblador, Guan: Guanaqueros, PTal: Punta Talca, LMol: Los Molles, Cura: Curaumilla, ECIM: Estación Costera de Investigaciones Marinas (Las Cruces), Pich: Pichilemu (see Fig. 1). Shaded boxes indicate values significant at the nominal level ($p < 0.05$). Values in **bold** indicate significant values after false discovery rate correction

A	CBaj	Apol	Temb	Guan	PTal	LMol	Mont	Cura	ECIM	Pich
CBaj	–	0.007	**0.013**	**0.011**	**0.009**	**0.022**	**0.025**	**0.023**	**0.023**	**0.020**
Apol	0.039	–	0.005	0.001	0.005	**0.016**	**0.021**	**0.019**	**0.017**	**0.012**
Temb	**0.065**	0.028	–	0.000	0.004	**0.020**	**0.009**	**0.027**	**0.015**	0.000
Guan	**0.053**	0.009	0.004	–	–0.005	**0.010**	0.005	0.008	**0.008**	0.004
PTal	0.043	0.031	0.023	–0.024	–	0.008	0.007	0.007	0.005	–0.001
LMol	**0.099**	**0.079**	**0.091**	0.043	0.031	–	**0.009**	0.002	**0.009**	0.005
Mont	**0.105**	**0.095**	0.038	0.020	0.024	0.036	–	0.004	–0.004	–0.002
Cura	**0.103**	**0.091**	**0.126**	0.030	0.023	0.009	0.017	–	0.003	**0.009**
ECIM	**0.090**	**0.071**	**0.059**	0.027	0.012	0.035	–0.013	0.015	–	–0.002
Pich	**0.086**	**0.057**	0.001	0.015	–0.004	0.024	–0.008	0.038	–0.005	–

B	CBaj	Apol	Temb	Guan	PTal	LMol	Mont	Cura	ECIM	Pich
CBaj	–	0.004	**0.010**	0.006	0.011	**0.016**	**0.020**	**0.023**	**0.018**	**0.019**
Apol	0.013	–	0.007	0.001	0.004	0.008	**0.015**	**0.013**	**0.012**	**0.010**
Temb	0.037	0.013	–	0.003	0.009	**0.013**	0.005	**0.024**	0.008	0.001
Guan	0.027	–0,009	–0.012	–	0.007	0.008	0.007	0.008	**0.010**	0.007
PTal	0.010	–0.002	–0.008	–0.045	–	0.011	**0.016**	**0.015**	0.010	0.010
LMol	0.073	0.054	0.060	0.016	0.007	–	0.007	0.004	0.009	0.005
Mont	0.079	0.070	0.018	0.005	0.001	0.019	–	0.007	0.000	0.000
Cura	0.084	0.063	0.105	0.010	0.002	–0.006	0.005	–	0.007	0.006
ECIM	0.068	0.052	**0.039**	0.016	–0.009	0.019	–0.022	0.001	–	0.000
Pich	0.061	0.035	–0.010	0.003	–0.026	0.003	–0.020	0.023	–0.021	–

Table 2. Results of generalized linear modeling (GLM) analyses employed to identify the best fit model for 5 variables explaining genetic diversity of *Notochthamalus scabrosus*. AIC: Akaike's information criterion, Ar: allelic richness; Gd: gene diversity; CHLA1(CHLA2): PC1 (PC2) of chlorophyll *a* concentration; SST1 (SST2): PC1 (PC2) of sea surface temperature; Cov: adult cover of *N. scabrosus* ($\log_{10}$ transformed); Lon: longitude, VarExp: variance explained. Values in **bold** are significant ($p < 0.05$)

GLM test			p	AIC	Test of effects		VarExp
Initial full model	Best fit models		p	AIC	Variable	p	(%)
Ar~CHLA1+CHLA2	Ar~CHLA2+Cov+Lon		0.568	19.38	CHLA2	**0.044**	48.61
+SST1+Cov+Lon					Cov	0.123	15.39
					Lon	0.436	3.74
	Ar~CHLA2+Cov		<**0.001**	18.47	CHLA2	**0.043**	48.61
					Cov	0.127	15.39
	Ar~CHLA2		<**0.001**	20.03	CHLA2	**0.025**	48.61
Gd~CHLA1+CHLA2	Gd~CHLA1+CHLA2+Cov		<**0.001**	-57.61	CHLA1	0.125	2.96
+SST1+Cov+Lon					CHLA2	**0.010**	67.80
					Cov	0.369	3.97
	Gd~CHLA1+CHLA2		<**0.001**	-58.15	CHLA1	0.173	2.96
					CHLA2	**0.005**	67.80
	Gd~CHLA2		<**0.001**	-57.31	CHLA2	**0.008**	67.80

878

879 Table 3. Results of the stepAIC analyses employed to identify the best fit model for 5
880 variables explaining genetic diversity of *Notochthamalus scabrosus*. Abbreviations as in
881 Table 2

stepAIC test		
Initial full model	Best fit model	AIC
Ar~CHLA1+CHLA2	Ar~CHLA2+Cov	22.69
+SST1+Cov+Lon		
Gd~CHLA1+CHLA2	Gd~CHLA1+CHLA2	–54.74
+SST1+Cov+Lon		

882

883 Table 4. Results of multiple regression on distance matrices (MRDM). Abbreviations as in
884 Table 2; values in **bold** are significant (p < 0.05)

MRDM full model		Coef	p	R^2	*F*	p
θ_{ST} ~ CHLA1+CHLA2+SST1+SST2	Int	0.007	0.512	0.55	6.445	**0.008**
+Rec+Cov+Lon	CHLA1	–0.006	0.534			
	CHLA2	–0.001	0.953			
	SST1	–0.041	0.629			
	SST2	0.024	**0.002**			

	Rec	0.004	0.401			
	Cov	−0.000	**0.036**			
	Lon	−0.014	0.084			
$D_{EST} \sim$ CHLA1+CHLA2+SST1+SST2 +Rec+Cov+Lon	Int	0.007	0.508	0.55	6.445	**0.007**
	CHLA1	−0.006	0.527			
	CHLA2	−0.001	0.951			
	SST1	−0.041	0.618			
	SST2	0.024	**0.002**			
	Rec	0.004	0.405			
	Cov	−0.000	**0.035**			
	Lon	0.078	0.078			

Fig. 1. Central-northern coast of Chile, showing the 10 sampling sites and weekly averages of sea surface temperature (SST) for nearshore areas. ECIM: Estación Costera de Investigaciones Marinas

Fig. 2. Principal coordinates analysis calculated by θ_{ST} (top) and D_{EST} (bottom) values of 10 sites studied. For θ_{ST} and D_{EST} indices, the first 2 axes explain 87.62 and 87.88% of the total variation, respectively. Site abbreviations as in Table 1

Fig. 3. STRUCTURE assignment of individual *Notochthamalus scabrosus* across all sites into clusters for *k* between 2 and 4. Colors indicate percentage contribution of individuals to assigned clusters (*y*-axis), individuals are represented by each line (*x*-axis); black lines separate sites from which individuals were collected. Site abbreviations as in Table 1

Fig. 4. Results for the linear regressions among 8 predictive and 2 dependent variables. Ar: allelic richness, Gd: gene diversity; CHLA1 (CHLA2): PC1 (PC2) of chlorophyll *a* concentration; SST1 (SST2): PC1 (PC2) of sea surface temperature; Rec: arrival rate of larval *Notochthamalus scabrosus* ($\log_{10}$ transformed); Cov: adult cover of *N. scabrosus* ($\log_{10}$ transformed); Lat: latitude; Lon: longitude. Star in panel f represents the Estación Costera de Investigaciones Marinas (ECIM) site
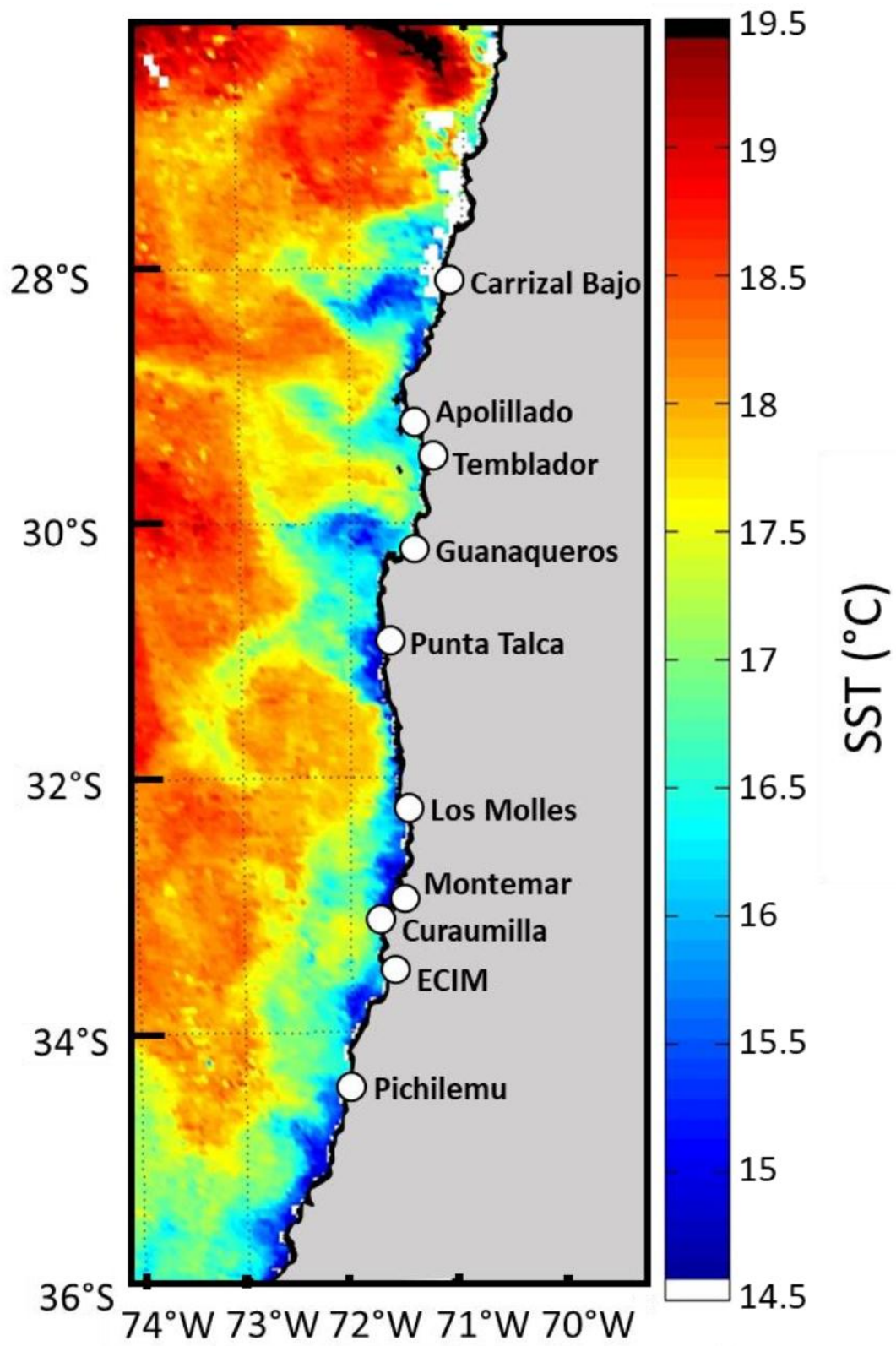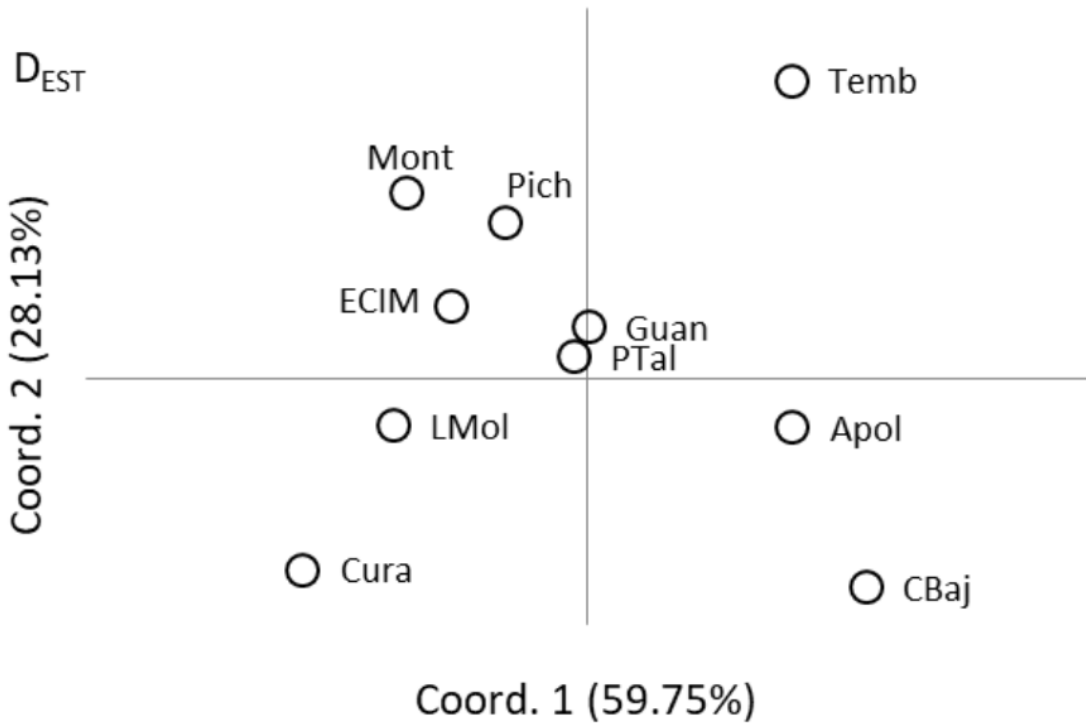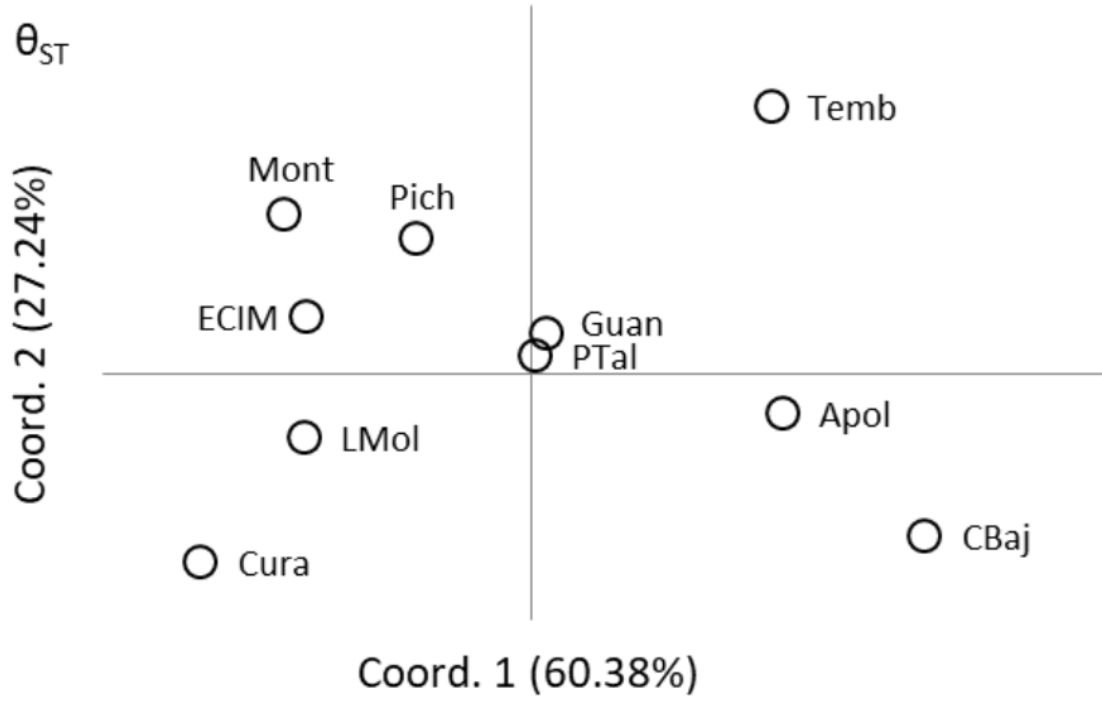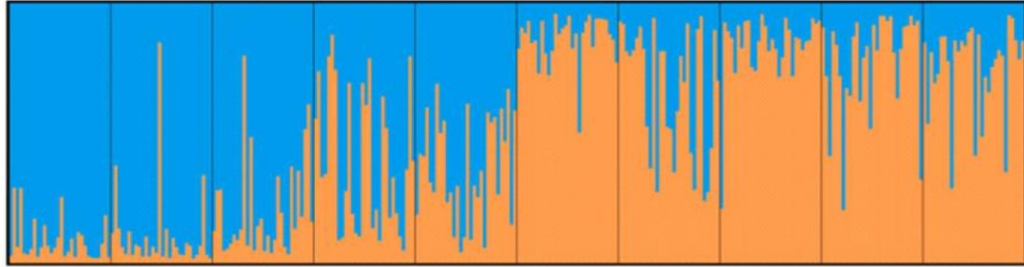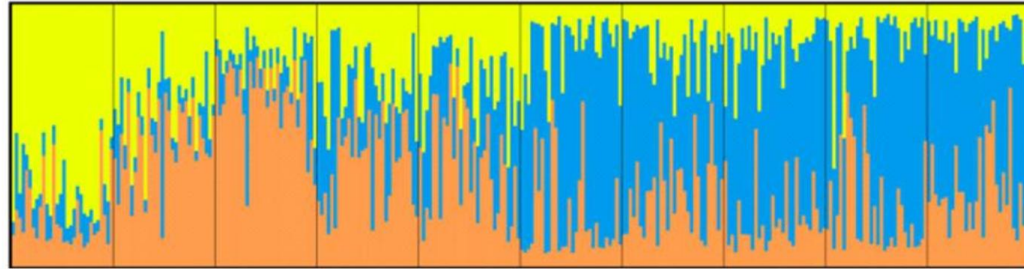
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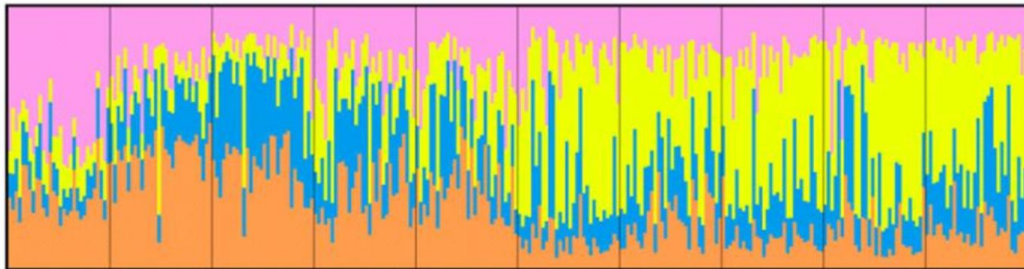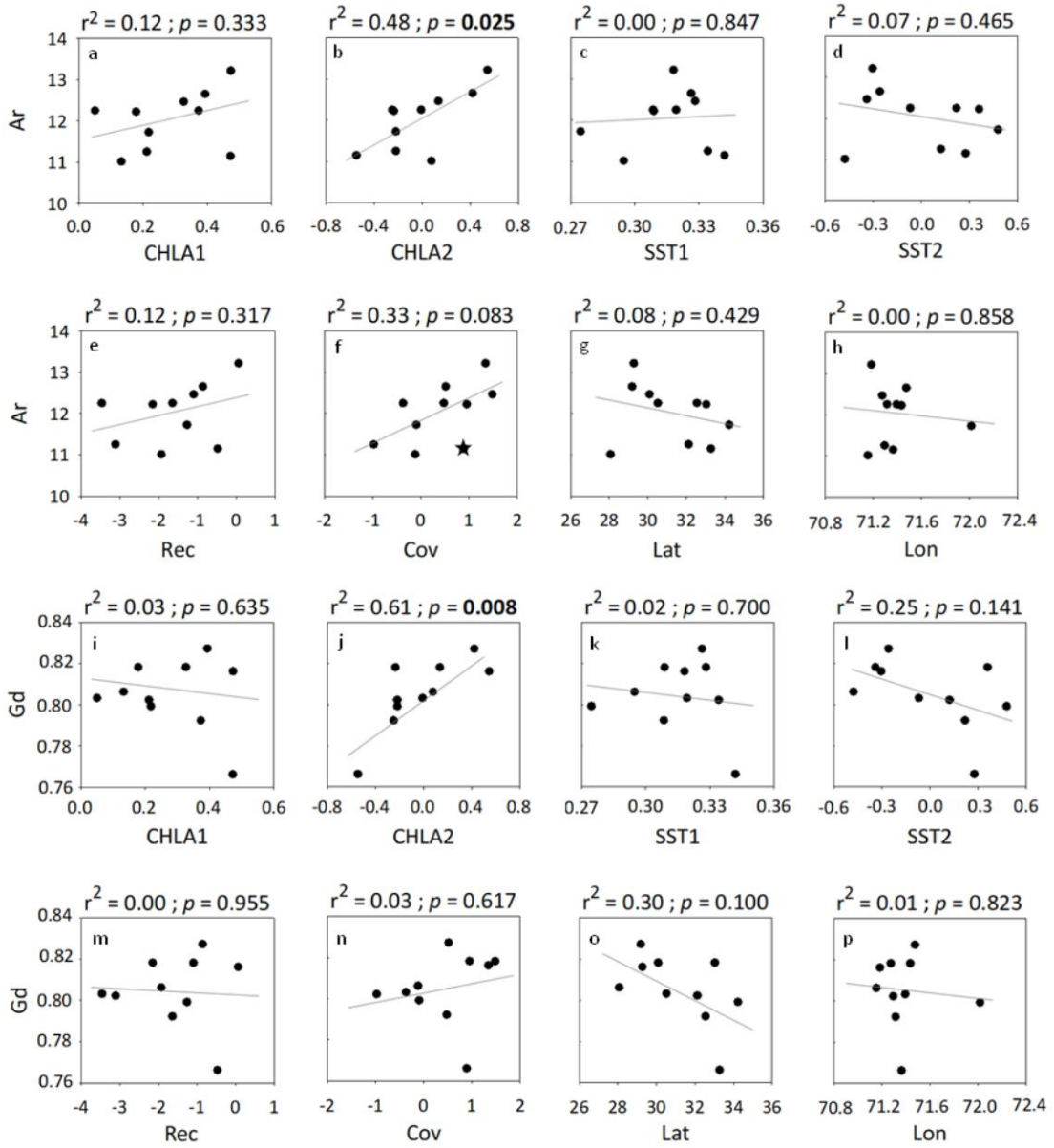
CBaj   Apol   Temb   Guan   PTal   LMol   Mont   Cura   ECIM   Pich

k = 3

CBaj   Apol   Temb   Guan   PTal   LMol   Mont   Cura   ECIM   Pich

k = 4
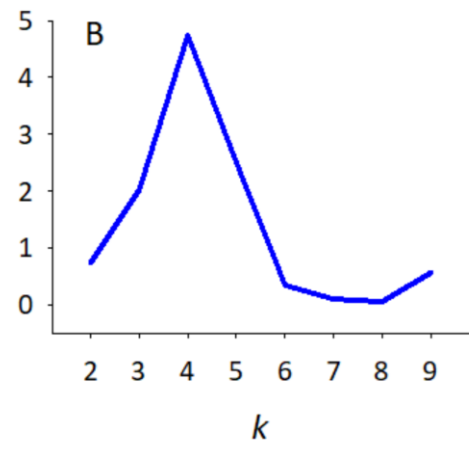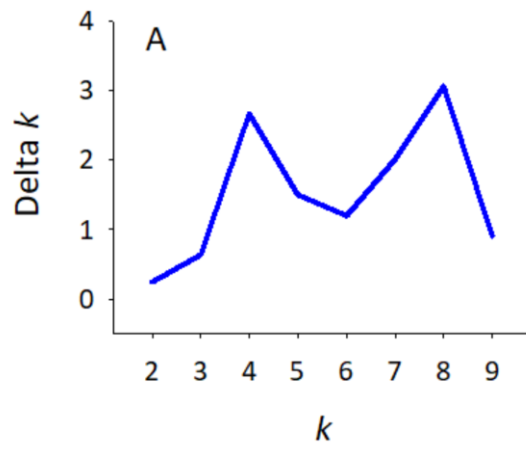
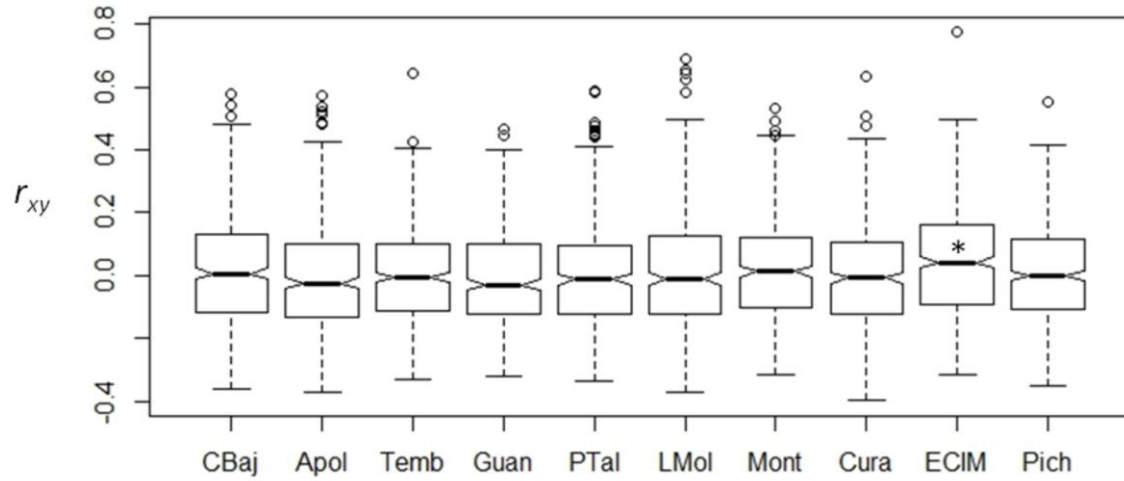CBaj   Apol   Temb   Guan   PTal   LMol   Mont   Cura   ECIM   Pich

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912   Figure S1

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915   Figure S2