Oceanographic heterogeneity influences an ecological radiation in elasmobranchs

Jonathan Sandoval-Castillo\textsuperscript{1,2}  |  Luciano B. Beheregaray\textsuperscript{1,2}  

\textsuperscript{1}Molecular Ecology Laboratory, College of Science and Engineering, Flinders University, Adelaide, SA, Australia  
\textsuperscript{2}Molecular Ecology Laboratory, School of Biological Sciences, Macquarie University, Sydney, NSW, Australia  

Correspondence  
Jonathan Sandoval-Castillo, Molecular Ecology Laboratory, College of Science and Engineering, Flinders University, Adelaide, SA, 5001, Australia. Email: jonathan.sandoval-castillo@flinders.edu.au  

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Abstract  
Aim: During ecological speciation, reproductive isolation is predicted to evolve between populations adapted to different biotic or abiotic environments despite the absence of geographical isolation. Regions of oceanographic heterogeneity (e.g. current interfaces, habitat transition zones, ecological gradients) are strong candidates for the presence of ecologically divergent natural selection, but their role in the radiation of elasmobranch species is yet to be tested. We used an integrative framework to assess the relative influence of oceanographic heterogeneity and geological history on the diversification of an elasmobranch genus.  
Location: Gulf of California (GC) and Baja California Peninsula (BCP), Mexico.  
Taxon: Shovelnose guitarfish (genus \textit{Pseudobatos}).  
Methods: We sampled 210 \textit{Pseudobatos} specimens from four distinct but physically connected oceanographic regions within the GC and in the BCP. We used genetic (mtDNA sequences and AFLP genotypes) and environmental (six oceanographic variables) datasets to clarify phylogenetic relationships, demographic history and evolutionary divergence among populations, and to test for associations between ecologically driven selection and reproductive isolation.  
Results: Phylogenetic and population genetic evidence exposed five distinct lineages of \textit{Pseudobatos} in the region, including four cryptic lineages in the GC. Phylogeographic analyses indicate a recent history of ecologically driven diversification associated with the Gulf’s young oceanographic environment and its four ecologically discrete regions. This hypothesis was supported by seascape genetics, ecological niche modelling and by tests of selection.  
Main conclusions: We propose an adaptive radiation for the genus \textit{Pseudobatos} linked with habitat heterogeneity of the GC. Our study likely represents the first assessment of an ecological radiation in the highly diverse elasmobranch group. It capitalizes on the environmental and biogeographic settings of the GC to offer a new perspective about the application of integrative approaches to study divergent natural selection and diversification in the sea.  

Keywords  
ecological speciation, evolutionary radiation, isolation by environment, marine biodiversity, phylogeography, seascape genomics
Many species appear to evolve by the process of ‘ecological diversification’ in which reproductive isolation evolves between populations adapted to different environments or ecological niches (Nosil, 2012; Schluter, 2009; Via, 2009). Theoretical and empirical evidence for ecological speciation has recently accumulated, challenging the dominant paradigm of physical mechanisms of diversification that underpin allopatric speciation (Beheregaray, Cooke, Chao, & Landguth, 2015; Teske et al., 2019). Nevertheless, links between diversification and natural selection are not always evident, and the role of ecological adaptation and divergence in the speciation process remains controversial (Nosil, 2012; Seehausen et al., 2014).

In marine ecosystems, spatial population disjunctions that result in speciation are often associated with vicariant events or with oceanographic discontinuities (Bowen, Rocha, Toonen, & Karl, 2013; Gaither, Toonen, Robertson, Planes, & Bowen, 2010). Regions of oceanographic heterogeneity (e.g., current interfaces, habitat transition zones, ecological gradients) are potentially strong candidates for the presence of ecologically based divergent selection between environments. Yet, the role of oceanographic heterogeneity as a driver of ecological diversification has not been satisfactorily addressed (Grummer et al., 2019; Riginos, Crandall, Liggins, Bongaerts, & Treml, 2016). Assessing geographical isolation and divergent natural selection in the ocean should benefit from studies of regional biota exposed to active geological history and complex oceanography. In this context, the Gulf of California (GC) and the adjacent Baja California Peninsula (BCP) provide an ideal study region. The geomorphological history of the Gulf has been particularly dynamic (Dolby et al., 2015; Umhoefer et al., 2018), and its current oceanographic conditions show high temporal and spatial variability (Ortega, Álvarez-Borrego, Arriaga, Renner, & Bridge, 2010). The processes underpinning the formation of the GC and the BCP are thought to have started ~12 million years ago (Mya), with the detachment of a proto-peninsula from the mainland and the formation of the southernmost GC (Dolby et al., 2015; Umhoefer et al., 2018). Tectonic activity transported the proto-peninsula and a volcanic archipelago 300 km north-west, allowing the flow of the northern GC ~6 Mya. At that time, the southernmost part of the GC was connected to the Pacific Ocean by seaways between islands. By ~3 Mya emerging land attached these islands and the proto-peninsula, closing the seaways and forming the BCP (Dolby et al., 2015; Murphy & Aguirre-Leon, 2002; Umhoefer et al., 2018). Tectonic activity transported the proto-peninsula and a volcanic archipelago 300 km north-west, allowing the flow of the northern GC ~6 Mya.

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Here, we used an analytical framework that integrates phylogenetics, phylogeography, genome scans and environmental modelling to assess the influence of geomorphological history and oceanographic heterogeneity on the diversification of *Pseudobatos* from the GC-BCP region. First, genetic divergence, migration rates, reproductive isolation and the delineation of cryptic lineages were assessed with phylogenetic and population genetic methods. Coalescent-based phylogeographic simulations were then conducted to test the fit of the genetic data to population histories predicted by competing evolutionary hypotheses. The latter is needed because ecological speciation usually requires a study system in which the existence of a dominant allopatric phase is unlikely in the context of evolutionary history (Endler, 1982). Finally, analyses based on seascape genetics, ecological niche modelling and tests for selection were used to assess oceanographic, ecological and geographical factors underpinning divergence. We predict that our integrative framework will detect associations between genetic and environmental divergence while accounting for the effects of geographical distance and historical biogeography (sensu, Beheregaray et al., 2015). We hypothesize that the *Pseudobatos* lineages from the GC-BCP have a recent history of ecologically driven diversification linked to oceanographically defined regions. This study capitalizes on the environmental and biogeographic settings of the GC-BCP to apply integrative genetic-based methods to study divergent natural selection and diversification in elasmobranchs.

### 2.2 | DNA analysis

We used a salting out protocol (Sunnucks & Hale, 1996) to extract genomic DNA. From 199 samples (178 shovelnose and 21 speckled guitarfish, Table S2), approximately 800 bp of the mtDNA control region (mtCR) was sequenced following Sandoval-Castillo and Beheregaray (2015). Nuclear data were generated for 183 samples (169 shovelnose and 14 speckled guitarfish, Table S2) using a modified protocol of amplified fragment length polymorphism (AFLPs; Zenger, Stow, Peddemors, Briscoe, & Harcourt, 2006) and loci determined using AFLPscore 1.4 (Whitlock, Hippierson, Mannarelli, Butlin, & Burke, 2008; locus threshold = 25%, phenotype-calling relative threshold = 10%). Error rate was assessed by running 24 samples twice from the DNA extraction step and using a mismatch error rate analysis in AFLPscore. Monomorphic loci (< 5% or > 95% all individuals) were excluded.

### 2.3 | Oceanographic dataset

Average annual oceanographic data of six key variables (temperature, salinity, oxygen saturation, nutrients concentration, total chlorophyll and bathymetry) for the last 100 years were obtained from the NOAA World Ocean Data Base (Boyer et al., 2018). Gridded maps at ~10 km² resolution of each oceanographic variable were generated using the DIVA algorithm in
ODV.5.2 (Schlitzer, 2015). These cover a quadrant from 103° to 117°W and 19° to 33°N that includes all sampling localities (Figure 1).

2.4 | Statistical analyses

2.4.1 | Phylogenetic and genealogical analyses

jModelTest 2 (Darriba, Taboada, Doallo, & Posada, 2012) was used to select the most appropriate substitution model for the mtCR dataset based on Bayesian information criterion. Phylogenetic analysis was performed using Bayesian inference in MrBayes 3.2 (Ronquist et al., 2012; replicates = 3, chains = 8, generations = 10,000,000, sampling every 100 generations, burn-in = 1,000,000). Genealogical relationships among mtCR haplotypes were inferred in TCS 1.2 (Clement, Posada, & Crandall, 2000; confidence connection=>95%).

2.4.2 | Genetic structure and demographic history

Genetic differentiation among localities was assessed by pairwise $\phi_{ST}$ (mtCR) in Arlequin 3.5 (Excoffier & Lischer, 2010) and $F_{ST}$ (AFLP) in AFLP-SURV 1.0 (Vekemans, 2002). Hierarchical population structure was tested for both the mtDNA and AFLP datasets (see Supporting Information) using an AMOVA in Arlequin. Here genetic variation was partitioned within sampled localities, among localities, and among mtCR lineages (PC, UG, IG, LG and Pg). Genetic structure and the most likely number of populations was assessed for the AFLP data with an approach for dominant markers in Structure 2.3.4 (Hubisz, Falush, Stephens, & Pritchard, 2009; admixture model with correlated alleles, burn-in = 100,000 iterations = 1,000,000). Historical demographic parameters ($\theta$ as population sizes, and $M$ as gene flow between populations) were estimated for the mtDNA data based on a full migration model using Migrate-N 3.6 (Beerli, 2006; slice proposal distribution, exponential with windows prior distribution, long chain = 1, burn-in = 10,000, run = 1,000,000, replicates = 7, static heating with five chains = 1, 1.5, 3, 6 and 100). Parameters were scaled by a mutation rate $\mu = 1.3 \times 10^{-07}$ based on an average substitution of $8 \times 10^{-3}$ substitutions per site per million years (Duncan, Martin, Bowen, & De Couet, 2006) and generation time of 16 years (Villavicencio-Garayzar, 1993). Finally, the time to the most recent common ancestor was inferred for all phylo-groups (i.e. major mtDNA lineages) using BEAST 1.7 (Drummond, Suchard, Xie, & Rambaut, 2012; standard priors, burn-in = 1,000, generations = 10,000,000, replicates = 5). Since a calibrated molecular clock is not available for Pseudobatos, we applied a widely used clock for sharks of 0.8% per million years (Corrigan, Huveeers, Schwartz, Harcourt, & Beheregaray, 2008; Dudgeon et al., 2012).
2.4.3 Detection of natural selection

Outlier AFLP loci under divergent selection were identified using two Bayesian $F_{ST}$-outlier approaches. First, we used Mcheza (Antao & Beaumont, 2011) to run pairwise comparisons between phylogroups. For each analysis null distributions of $F_{ST}$ were generated using 50,000 simulations, a $\alpha$ of 0.01, and a confidence interval of 99%. Loci identified in more than two comparisons were considered outliers (Campbell & Bernatchez, 2004). BayeScan 2.1 (Foll & Gaggiotti, 2008) was also used to calculate $F_{ST}$ coefficients and decompose them into a locus-specific component ($\alpha$) and a population-specific component ($\beta$). Only those loci with very strong evidence for selection ($\text{log}10BF > 1.5$) were considered (Jeffreys, 1961). To further reduce false positives, only loci classified as outliers by both methods were considered as candidate loci for selection.

2.4.4 Ecological isolation

Environmental distances were estimated as the difference of each oceanographic variable between sampling sites. Geographical distances between sites were measured along the coastline using GoogleEarth (2017). We then used SAM (Joost, Kalbermatten, & Bonin, 2008) to calculate multiple univariate logistic regressions to test for association between allele frequency of each of the 541 AFLPs and the five environmental variables. Associations were considered significant only if both G and Wald tests rejected the no-association model at a 2.3E-9 threshold. In addition, the contribution of geographical and environmental factors to inferred genetic patterns, and the correlations between genetic structure (i.e. measured by mtDNA $\Phi_{ST}$ and AFLP $F_{ST}$) and geographical and environmental distances were assessed using partial Mantel tests in IBSWS 3.23 (Jensen, Bohonak, & Kelley, 2005; permutation = 10,000). We also used a partial redundancy analysis (RDA) to assess genotype-environmental associations between the five oceanographic variables and the allele frequencies of the candidate loci. The best model was selected using a backwards-stepwise selection procedure. Then the significance of the final model and each environmental variable was assessed by 1,000 ANOVA permutations. To determine the extent of ecological overlapping between taxa (i.e. phylogroups), predictive distribution models were generated using maximum entropy in MaxEnt 3.4.1 (Phillips, Anderson, Dudík, Schapire, & Blair, 2017). This predicts taxa environmental suitability of each cell in a gridded map. Two or three commercial fisheries sites per locality (9–15 per taxon) were used as the taxa presence dataset, and gridded maps of six oceanographic variables were used as environmental layers. A receiver operating characteristic (ROC) analysis was performed and the area under the ROC curve (AUC) was used to evaluate the discrimination power of the predicted distribution (Phillips & Dudík, 2008). In addition, symmetric extremal dependence indices (SEDIs) were calculated using the R package MAXENTOOLS 1.1.0 (Scavetta, 2019). Niche overlap among phylogroups was quantified using niche similarity indices ($I$) and tested using identity tests in ‘ENMTools’ 0.2 (Warren, Glor, & Turelli, 2010). For the identity test, a null distribution of overlapping score between taxa was generated based on 1,000 occurrence point pseudoreplicates per taxon. Niche identity was rejected when the actual observed $I$ index was significantly lower than that expected for the pseudo-replicated dataset.

2.4.5 Assessing alternative scenarios of evolutionary divergence

DIYABC 2.1.4 (Cornuet et al., 2014) was used to test competing phylogeographic hypotheses (Figure 2). Results from Migrate-N were used to set each extant population size. Major historical geological events were used to set putative splitting times: (a) the end of the formation of the gulf 6 Mya (Oskin & Stock, 2003), (b) the opening of a seaway crossing Baja California in the southern part of the gulf 3 Mya (Helene & Carreño, 1999), and (c) the opening of a putative seaway at mid-peninsula 3 Mya (Ochoa-Landin, Ruiz, Calmus, Pérez-Segura, & Escandon, 2000) or 1 Mya (Murphy & Aguirre-Leon, 2002). We tested 11 hypotheses (Figure 2c): three that include vicariance and ecological radiation events (H1a; H1b; H1c), six that consider only the vicariant events (H2a; H2b; H2c; H2d; H4a; H4b) and two considering ecological radiation only (H3a; H3b) (details in Supporting Information). For each evolutionary model, 1,000,000 datasets were simulated using the priors in Table S1. Subsequently, 60 summary statistics were calculated for each scenario and the empirical dataset. The probability of each scenario was calculated using logistic regression of simulated datasets against the linear discrimination components of the empirical data. We then calculate both type I and II error rates per scenario (see Supporting Information).

3 RESULTS

3.1 Genetic diversity and loci classification

Fifty-two mtCR haplotypes and 541 nuclear loci were resolved for all samples. Haplotype diversity was moderate to high (0.551 to 0.952). All nuclear loci were polymorphic with expected heterozygosity ranging from 0.313 to 0.407 (Table S2). Of the 541 nuclear loci, 39 candidates for selection (7.2%) were detected by both outlier methods (Mcheza detected 68 loci and BayeScan 44; Table S3).

3.2 Cryptic genetic diversification of Pseudobatos lineages

Nuclear and mitochondrial data provided strong evidence for five lineages of Pseudobatos in the GC-BCP region. One lineage (Pg) corresponds to all organisms identified in the field as P. glaucostigma. Samples originally identified as P. productus comprised four discrete lineages that show strong ecological differentiation. Each lineage was found in a different oceanographically delimited ecological
region, namely the UG, Islands (IG), PC and LG (Figure 2d,e). In contrast, the \textit{P. glaucostigmus} lineage occurs in both the OG and in the LG, in sympatry with the LG cryptic lineage of \textit{P. productus}. The inferred lineages comprise five moderately supported clades (> 0.78 Bayesian support), with four (LG, Pg, UG and PC-IG) being reciprocally monophyletic (Figure 3a). Phylogenetic subdivision is reflected in strong genetic structure across the 16 sites (mtDNA $\Phi_{ST} = 0.876$, $p < .01$; AFLP $F_{CT} = 0.139$, $p < .01$; $\Phi_{PT} = 0.282$, $p < .01$). AMOVA shows that molecular variation is best explained by differences between bioregions (mtDNA $\Phi_{CT} = 0.869$, $p < .01$; AFLP $\Phi_{RT} = 0.244$, $p < .01$; Table S4). Bayesian clustering analyses suggest heterogeneous genomic divergence: Neutral loci showed moderate differentiation and substantial admixture between lineages; on the other hand, the 39 AFLP candidate loci showed complete differentiation and nil or very low admixture (Figure 3b; $K = 5$). Here, all individuals were allocated accurately to each mtCR lineage with very little to no nuclear gene flow or introgression between them (Figure 3b). This agrees with estimates of historical migration in which all pairwise migration parameters ($m$) from Migrate-N point to a higher probability distribution near zero (Table S5). Overall, the inferred levels of genetic and genealogical differentiation suggest that the five \textit{Pseudobatos} lineages are mostly reproductively isolated.

### 3.3 Hypothesis testing of an ecological radiation

Out of the 11 hypotheses assessed, DIYABC results supported with high confidence the hypothesis of a single and recent ecological radiation (H3a, $p > .86$; Figure 4; Table S7).

### 3.4 Ecological isolation and niche modelling

There was statistical support for correlations between genetic distance and nutrient concentration, oxygen saturation, and bathymetry, even after controlling for the effect of geographical distance (Table 1). Allele distributions of 57 (10.5%) of 541 AFLP loci were significantly associated with at least one oceanographic variable.
Furthermore, 26 of these loci were also candidate loci detected by both outlier analyses (Table S3), and environmental variation explained 68.2% of the genetic variation present in the candidate loci \( (p < .001; \text{Figure S4}) \).

Environmental niche modelling showed high entropy with nil to moderate geographical overlap (Figure 3c). Niche identity was rejected for all pairs of lineages, with moderate \( I \) index values, except for LG versus Pg (Table S6). The latter lineages are sympatric and show high niche overlap.

### DISCUSSION

Diversification in elasmobranchs has been traditionally associated with vicariant biogeographic history and dispersal limitations (Dudgeon et al., 2012; Musick et al., 2004; Sandoval-Castillo, 2019). Our work showcases the role of isolation by environment in generating and maintaining diversity in this group and suggests that mobility might not hinder speciation in sharks and rays. This adds to cumulative evidence of the importance of heterogeneous marine
environments in shaping ecological and evolutionary divergences in species with relatively moderate to high dispersal potential (e.g. Pazmiño, Maes, Simpfendorfer, Salinas-de-León, & van Herwerden, 2017; Sandoval-Castillo, Robinson, Hart, Strain, & Beheregaray, 2018; Teske, Sandoval-Castillo, Waters, & Beheregaray, 2017).

4.1 Multiple cryptic species of Pseudobatos in the Gulf of California and adjacent coast

A range of analytical methods based on mtDNA and nuclear data provided strong evidence for five lineages of *Pseudobatos* in the GC-BCP region, including four cryptic lineages within *P. productus*. These show strong concordance with oceanographically delimited ecological regions (Figure 2d,e). The UG lineage of *P. productus* appears more closely related to the morphologically distinct *P. glaucostigma* (Figure 3b). The other *P. productus* lineages (LG, PC and IG) are also highly divergent and show nil historical gene flow with other *Pseudobatos* lineages from the region. It is conceivable that the LG lineage might represent the spiny guitarfish *P. spinosus*, a taxon described based on a single museum specimen (Günther, 1870). Although morphological data from three specimens suggest that *P. spinosus* occurs in one locality from the GC (Castro-Aguirre & Pérez, 1996), this taxon has since been synonymized with *P. productus* (Compagno, 2005). There are no described taxa or synonyms that could be associated with the other cryptic lineages, but reproductive data suggest the existence of distinctive lineages in the GC and the PC (Romo-Curiel, Sosa-Nishizaki, Pérez-Jiménez, & Rodríguez-Medrano, 2017; Villavicencio-Garayzar, 1993). Delimiting species on the basis of genetic data can be controversial, but the phylogenetic distinction and levels of genetic differentiation depicted here, in conjunction with results about niche partition and ecological divergence, satisfy a number of different properties used to delineate species. These include operational criteria used in species concepts such as the biological (Dobzhansky, 1950; Mayr, 1942) and some versions of the phylogenetic (Nelson & Platnick, 1981) concept. Thus, a total of four cryptic lineages with likely different stages of evolutionary separation are reported here for *P. productus* in the GC-BCP region.

4.2 Population history and hypothesis testing of an ecological radiation

Our results suggest a combined impact of the BCP on initial isolation of *Pseudobatos* lineages (PG vs. GC), followed by an ecological radiation at around 1 Mya within the GC. Adaptive radiations are prompted by ecological opportunities in scenarios where incipient lineages are exposed to a wealth of evolutionarily accessible habitats and resources (Kennedy et al., 2017; Losos, 2010; Pontarp & Wiens, 2017). Historical environmental changes and associated range expansions (e.g. Hewitt, 2000) have resulted in opportunities for adaptive divergence and niche specialization (Rodrigues & Diniz-Filho, 2016). The establishment of the present-day heterogeneous environmental setting of the GC took place between ~2 and 1 Mya, after the final formation of the BCP and the main bathymetric features of the GC (García Sánchez et al., 2019; Mark et al., 2017; Sutherland et al., 2012). We hypothesize that these events, in combination with the mid-Pleistocene transition (1.2–0.8 Mya; Chalk et al., 2017), created ecological opportunities that likely promoted adaptive phenotypic differences between the lineages of *Pseudobatos*. Despite the inferred strong genetic and ecological divergence (see below), there are no known conspicuous adaptive traits in morphology between *Pseudobatos* lineages from the GC (Marquez-Farias, 2007; Romo-Curiel et al., 2017). Morphological stasis through different stages of cladogenesis has been reported for several groups (Barley, White, Diesmos, & Brown, 2013; Beheregaray & Caccone, 2007; Bickford et al., 2007; Van Boxel & Hunt, 2013), including elasmobranchs (Ebert & Compagno, 2007; Jones et al., 2017; Last, 2007). One possibility is that rates of morphological diversification in elasmobranchs are limited by the interaction of genetic and developmental constraints, as in some invertebrates (Appeltans et al., 2012; Beldade, Koops, & Brakefield, 2002; Eldredge et al., 2005). Alternatively, the apparent morphological stasis could be due to relatively low niche differentiation within the Gulf (Cothran, Henderson, Schmidenberg, & Relyea, 2013; Scriven, Whitehorn, Goulson, & Tinsley, 2016). The adaptive traits involved in this radiation could also have a physiological basis. In fact, a transcriptomic study of a teleost group has shown that physiological traits linked to environmental tolerances can be under strong selection and delimit sister species ranges across climatic gradients (Sandoval-Castillo et al., 2019). Understanding whether elasmobranch radiations respond by becoming less morphologically diverse in scenarios of relatively limited ecological opportunity (e.g. Corrigan & Beheregaray, 2009), either because of limited developmental paths or due to physiological adaptations, requires currently unavailable phenotypic data. These data, in conjunction with refined statistical approaches that directly link the tempo of lineage diversification to the tempo of phenotypic evolution (Moen & Morlon, 2014) are needed to assess the adaptive nature of the radiation and to conduct a taxonomic revision of *Pseudobatos*.

4.3 Ecological adaptation and speciation

The first requirement for ecological speciation is a source of divergent selection, and some of the main triggers of divergent selection are habitat structure or contrasting niches (Schluter, 2003). The predicted niche models provide support for ecological partition among *Pseudobatos* lineages and are congruent with habitat heterogeneity and structure proposed for the GC with respect to productivity, oxygen, salinity, temperature, nutrients and bathymetry (Ortega et al., 2010). We also detected contrasting patterns of differentiation between the neutral and candidate loci datasets. Analyses with neutral data showed relatively moderate admixture between *Pseudobatos* lineages and suggests some level of gene flow. During early stages of speciation (as
expected for *Pseudobatos* in the GC), gene flow can prevent the evolution of adaptive divergence (Via, 2012). However, substantial population divergence and rapid speciation between ecologically dissimilar populations in the face of ongoing gene flow has been reported for several organisms (e.g. Beheregaray & Sunnucks, 2001; Bernatchez et al., 2010; Cooke, Chao, & Beheregaray, 2012; Cooke, Landguth, & Beheregaray, 2014; Sandoval-Castillo et al., 2018). On the other hand, *Pseudobatos* lineages are completely differentiated based on candidate loci (Figure 3b). Long-term reproductive isolation facilitates the accumulation of neutral divergence that could be confused with genetic differentiation associated with traits under selection (Rundle & Nosil, 2005; Via, 2009). However, the Bayesian analyses suggest a relatively short time to the most recent common ancestor of *Pseudobatos* lineages (< 1.5 Mya; Figure 3a), a scenario consistent with the young age of the heterogeneous environment of the GC. In addition, heterogeneous genomic divergence has been associated with variation in recombination rates during the process of adaptive diversification with gene flow (Roesti, Hendry, Salzburger, & Berner, 2012; Tine et al., 2014). Thus, we consider that *Pseudobatos* in the GC-BCP exemplifies a radiation involving divergent natural selection with gene flow.

Genetic–environmental associations provide additional evidence for divergent selection that might eventuate in ecological speciation (Bierne, Welch, Loire, Bonhomme, & David, 2011; Stucki et al., 2017). Here, positive trends between genetic distance and three oceanographic variables remained significant after controlling for geographical distance (Table 1). Allele distributions of 26 of 39 outlier loci were significantly associated with at least one oceanographic variable (Table S3; Figure S4). Moreover, the inferred ecological isolation among *Pseudobatos* lineages (Figure 3c; Table S6) suggests an important role of natural selection in their diversification. The different oceanographic dynamics create sharp dissolved oxygen and nutrients concentration gradients between bioregions in the GC, with some hypoxic and nearly anoxic areas in the LG and OG and highly productive areas in the UG, IG and LG (LLuch-Cota et al., 2007). We propose that the development of metabolic specializations associated with differences in oxygen concentration and diet along ecologically distinct bioregions influenced the radiation of *Pseudobatos* in the GC. Oxygen consumption requirements differ between species of elasmobranchs (Speers-Roessch et al., 2012), and low oxygen saturation creates selective pressure for physiological adaptations (Renshaw, Wise, & Dodd, 2010; Routley, Nilsson, & Renshaw, 2002). Although nutrient concentration may not directly affect predators such as elasmobranchs, this factor is strongly correlated with primary productivity, which in turn affects abundance and diversity of prey (Korpinen, Jormalainen, & Pettay, 2010). In fact, a key feature of the evolutionary success of elasmobranchs relates to their ecological and anatomical feeding specializations that allowed them to radiate into numerous niches (Ferguson, Higdon, Tallman, Fisk, & Hussey, 2014; Walter et al., 2017; Wilga, Motta, & Sanford, 2007).

The lack of conspicuous morphological differences among lineages suggests that sexual selection might be unlikely. However, divergent habitat preferences are known to generate reproductive isolation if mating occurs in the preferred habitat (Rundle & Nosil, 2005), which can be considered as a type of sexual selection (Oring & Lank, 1982). Species of *Pseudobatos*, including *P. productus*, show strong habitat preferences for reproduction (Márquez-Farías, 2007; Villavicencio-Garayzar, 1993). These can be manifested as fidelity of some individuals to mating locations (reproductive philopatry), a driver of genetic structure in several elasmobranchs (Corrigan, Huveneers, Stow, & Beheregaray, 2015; Portnoy & Heist, 2012; Sandoval-Castillo & Beheregaray, 2015). For instance, for bonnet-head sharks (*Sphyrna tiburo*), philopatry was suggested to facilitate sorting of locally adaptive genetic variation among locations and environments (Portnoy et al., 2015). Philopatric behaviour can be a form of prezygotic sexual selection and that might contribute to the patterns observed here (Nosil, Funk, & Ortiz-Barrientos, 2009). We hypothesize that divergent habitat preferences for reproduction, potentially linked to gradients in oxygen, nutrients and bathymetry, have influenced the evolution of reproductive isolation among *Pseudobatos* lineages in the GC.

A limitation of our study is that genome scans were based on anonymous AFLP dominant markers. Genome-wide marker panels, whole genomes and transcriptomes can now be used to better delineate barriers to gene flow, selection gradients and isolation by environment, and to identify gene regions and phenotypic traits involved in adaptation (Grummer et al., 2019; Rellstab, Gugerli, Eckert, Hancock, & Holderegger, 2015; Riginos et al., 2016; Sandoval-Castillo, 2019). Regional-scale studies in sharks and rays should test for population diversification across heterogeneous environments while controlling for spatial genetic autocorrelation and vicariant biogeographic history (Beheregaray et al., 2015). These surveys would also benefit from a priori spatial delineation of adaptive phenotypes. Such studies are expected to contribute substantially to integrative taxonomic efforts (Dayrat, 2005) and to the conservation and management of elasmobranchs worldwide (Sandoval-Castillo, 2019).

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DATA AVAILABILITY STATEMENT

AFLP genotypes, mtDNA sequences, sample coordinates and environmental data used in analyses are available on Figshare Digital Repository (DOI: 0.6084/m9.figshare.10317203) and on GenBank (accession numbers: MT134048-MT134099).
REFERENCES


