



Multilocus phylogeography of the endemic and endangered angular angelshark (*Squatina guggenheim*) in the Southwest Atlantic Ocean

Ingrid Vasconcellos Bunholi · Bruno Lopes da Silva Ferrette · Rodrigo Rodrigues Domingues · Matheus Marcos Rotundo · Juan Martín Cuevas · Mirta García · Sebastián Gómez · Renato Hajenius Aché de Freitas · Claudio Oliveira · Fausto Foresti · Fernando Fernandes Mendonça

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Abstract The angular angelshark (*Squatina guggenheim*) is a coastal endangered angel shark from the Southwest Atlantic Ocean and one of the major bycatch victims. Despite major concerns about this species, little is known about its evolutionary connectivity across its whole geographic distribution. Here, genetic connectivity and phylogeographic patterns of *S. guggenheim* for 122 individuals were

assessed across the Southwest Atlantic Ocean regions based on a multilocus mitochondrial DNA approach to support conservation strategies. The concatenated mitochondrial dataset (control region, cytochrome b and cytochrome c oxidase I) showed high levels of haplotype diversity and low nucleotide diversity in *S. guggenheim*, with distinct genetic diversity patterns among populations. Although signs of stepping-stone gene flow were observed, a strong and statistically significant genetic structure into at least two populations was detected, matching with the species' biological traits and region's oceanographic particularities. Contrasting demographic patterns were detected,

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I. V. Bunholi (✉) · B. L. da Silva Ferrette · R. R. Domingues · F. F. Mendonça
Laboratório de Genética Pesqueira e Conservação (GenPesC), Instituto do Mar, Universidade Federal de São Paulo (UNIFESP), Campus Baixada Santista, São Paulo, Brasil
e-mail: ingridbunholi@gmail.com

I. V. Bunholi · C. Oliveira · F. Foresti
Laboratório de Biologia e Genética de Peixes, Instituto de Biociências de Botucatu, Universidade Estadual Paulista Júlio de Mesquita Filho (UNESP), Bauru, Brasil

I. V. Bunholi
Department of Biology, Indiana State University, Terre Haute, IN, USA

B. L. da Silva Ferrette
Laboratório de Genética da Conservação, Universidade Santa Cecília (UNISANTA), Santos, SP, Brasil

M. M. Rotundo
Laboratório de Pesquisas Biológicas (LAPEBio), Universidade Santa Cecília (UNISANTA), Acervo Zoológico, Santos, Brasil

J. M. Cuevas · M. García · S. Gómez
Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata, La Plata, Argentina

J. M. Cuevas
Wildlife Conservation Society de Argentina, Amenábar 1595, P. 2, Of. 19, CABA, Argentina

R. H. A. de Freitas
Laboratório de Biologia de Teleósteos e Elasmobrânquios (LABITEL), Departamento de Ecologia e Zoologia, Universidade Federal de Santa Catarina (UFSC), Florianópolis, Brasil

in which only southernmost Atlantic populations showed signs of population expansion. Despite the existence of connectivity among regions, our results suggest that conservation plans should be carried out following the uniqueness of each management unit.

Keywords Conservation genetics · Elasmobranch · Genetic diversity · Mitochondrial DNA · Population structure · Southwest Atlantic

Introduction

Understanding the patterns of genetic population structure of a species depends on a variety of micro-evolutionary approaches and environmental factors over time (Avice, 2009). This knowledge can be reached through phylogeographical studies from which genetic surveys and biogeographic traits must be connected (Avice, 2009). The current marine biodiversity is the reflection of a long-term evolution, which has been accessed to understand the dynamics of population connectivity and phylogeography (Bowen et al., 2014). Contemporary populations can often carry genetic signatures from past climatic changes, geographic and geological history (Felsenstein, 1982; DiBattista et al., 2011). Several variations in environmental conditions occurred in the last 2.58 million years ago (Mya) during the glacial-interglacial cycles of the Quaternary Period throughout the world's oceans, such as sea-level declines, sea temperatures oscillation and marine currents changes (Rabassa et al., 2005; Ludt & Rocha, 2015; Alves & Mahiques, 2019). As a consequence, coastal marine and estuarine species were deeply affected (e.g., Beheregaray et al., 2002; Iriarte et al., 2011; Machado et al., 2017) and this period has been known as an important driven factor for evolutionary variability and changes in species distribution (Hewitt, 2000).

Specifically, the Southwest Atlantic Ocean is one of the most biologically productive areas of the world with relatively high species richness and distinct temperatures throughout the coastline (Acha et al., 2004; Lutz et al., 2010; Franco et al., 2020). Despite its high biodiversity, the Southwest Atlantic Ocean is remarkably vulnerable to unsustainable fishery efforts (Lucifora et al., 2012). As respects to the Chondrichthyes class (comprising sharks, rays and chimeras), 50% of endemic species from the Southwest Atlantic are

threatened with extinction (Dulvy et al., 2014). Even though the number of conservation genetic studies have increased in the past few decades, many endemic Chondrichthyes from the Southwest Atlantic Ocean are still data deficient (Domingues et al., 2018). Therefore, estimating the number of populations as well as the process and patterns that have been shaping the genetic structure of a species over time is imperative to guarantee a short and long-term conservation of threatened shark species (Allendorf et al., 2013).

The angular angelshark *Squatina guggenheim* Marini, 1936 is a small marine demersal shark, endemic from the continental shelf of the Southwest Atlantic Ocean. Its distribution ranges between Rio de Janeiro in Brazil and central Patagonia in Argentina, inhabiting sandy and muddy substrates at depths of 10–80 m (Cousseau & Figueroa 2001; Ebert et al., 2013; Cuevas et al., 2020). As most part of benthic elasmobranchs, *S. guggenheim* presents low dispersal capability, resulting in restricted reproductive migrations even at small geographic scales (Colonello et al., 2007). Furthermore, the females have a three-year reproductive cycle that determines that migration movements related to mating and parturition do not affect all the reproductive population (Vooren & Klippel, 2005). *Squatina guggenheim*, jointly with two others endemic angel sharks from the Southwest Atlantic, *Squatina occulta* Vooren & da Silva, 1992 and *Squatina argentina* Marini, 1930, has been regularly caught as a valuable bycatch in coastal trawlers and gillnet fisheries in southern Brazil, Uruguay and northern Argentina (Chiaromonte, 1998; Vooren & Klippel 2005; Bunholi et al., 2018; Oddone et al., 2019). Despite the implementation of conservation laws controlling local fisheries throughout the Brazilian coast since 2004, capture and illegal trade of angel sharks were recently documented (Bunholi et al., 2018). Hence, intensive fishery pressure coupled with the low reproductive potential makes this species highly vulnerable to extinction (Dulvy et al., 2008). As a consequence, *S. guggenheim* is currently listed as Endangered by the International Union for Conservation of Nature (IUCN) with a decreasing population trend (Oddone et al., 2019). Regionally, the species is reported as Critically Endangered in the Brazilian regional conservation status classification (ICMBio/MMA, 2018; Vooren

et al., 2018) and Endangered for the Patagonian Sea (Cuevas et al., 2020). To date, the single genetic study that evaluated the population genetic structure of *S. guggenheim* revealed no latitudinal structure across the region of the Rio de La Plata (García et al., 2014). Yet, the lack of sampling from Brazil, therefore, does not provide enough information to characterize its population structure.

The conditions for the dispersion of individuals, in most cases, are correlated to geographical barriers to gene flow as well as biological characteristics (Avise, 2000; Hellberg et al., 2002; Ovenden, 2013; Hirschfeld et al., 2021). The Southwest Atlantic continental shelf is strongly influenced by the Brazilian subtropical and the Malvinas-Falklands subantarctic currents, which together had originated the subtropical convergence between 30°S and 40°S (Piola et al., 2000; Odebrecht & Castello, 2001; Acha et al., 2004). In addition to variations of temperature, an intense salinity variation occurs in that region due to the presence of large freshwater discharges that produces a low density and stable water layer over the Patos Lagoon (32°S) and Rio de la Plata (35°S) setting up an extended estuarine influence of 50 km and 240 km, respectively (Odebrecht & Castello, 2001; Acha et al., 2004). However, despite having complex oceanographic characteristics that may restrict connectivity, those are considered permeable barriers and may not represent significant breaks in connectivity (Ovenden, 2013; Hirschfeld et al., 2021). Several authors have suggested that the permeable barriers of the Southwest Atlantic Ocean (ocean currents and fresh-water discharges) do not affect connectivity of marine animals (Iriarte et al., 2011; Cortinhas et al., 2016; Machado et al., 2020). For example, no genetic isolation between Argentina and southern Brazil was observed for *Pogonias courbina* Lacepède, 1803, likely resulting from its reproductive strategy and seasonal migrations across the Brazil-Malvinas confluence zone (Machado et al., 2020). On the other hand, other studies suggest that those oceanographic particularities can affect the genetic structure of marine species (Santos et al., 2006; Rodrigues et al., 2014; Fruet et al., 2017; Márquez et al., 2017). Furthermore, in elasmobranchs, life history characteristics also influence dispersal events and, consequently, the geographical patterns of genetic variation (Hirschfeld et al., 2021). This influence was observed in two species of guitar fishes that showed high genetic

population structure in the Southwest Atlantic Ocean, which might be also due to their resident behavior with only seasonal depth migrations (Cruz et al., 2021).

Against this background, a multilocus dataset was used to infer the genetic connectivity and phylogeographic aspects of *S. guggenheim* in the Southwest Atlantic Ocean. In particular, the objectives of this study were: (i) to describe the patterns of genetic diversity of *S. guggenheim* in the southwest Atlantic Ocean; (ii) to test the null hypothesis of panmixia in this region; and (iii) to identify the evolutionary process responsible for shaping the current pattern of distribution and demography of the species.

Materials and methods

Sampling and DNA sequencing

A total of 122 angular angelshark *Squatina guggenheim* tissue samples (muscle or fin clip) were opportunistically obtained between 2015 and 2018 from industrial bottom trawlers, gillnets, longline and artisanal fisheries across its distribution in the Southwest Atlantic Ocean. Sampling regions: Northern Coast of São Paulo State, Brazil—NCSP (n = 7); Baixada Santista São Paulo State, Brazil—BSSP (n = 37); Southern Coast of São Paulo State, Brazil—SCSP (n = 41); Southern Brazil—SOBR (n = 16) and Coast of Uruguay-Argentina—UYAG (n = 21—which includes Uruguay—Punta del Este region—UYAG1 and Argentina—Villa Gesell region—UYAG2) (Fig. 1). The reason that we decided to merge Uruguay and Argentina as UYAG region is due to the low number of samples from Uruguay—Punta del Este region (UYAG1) as well as the non-significant genetic variance between both regions (see Fig. 1 Appendix 1 in Supplementary Material for details). All tissue samples were stored in 95% ethyl alcohol.

Total genomic DNA was extracted using the DNeasy Blood and Tissue Kit (QIAGEN®, Valencia, CA, USA). Polymerase Chain Reaction (PCR) was performed for each mtDNA molecular markers: non-protein-coding control region (CR) and protein-coding genes cytochrome *b* (CytB) and cytochrome *c* oxidase I (COI) following the standard protocol of Platinum™ Taq DNA Polymerase (Invitrogen™) (see Appendix 1 in Supplementary Material for details).

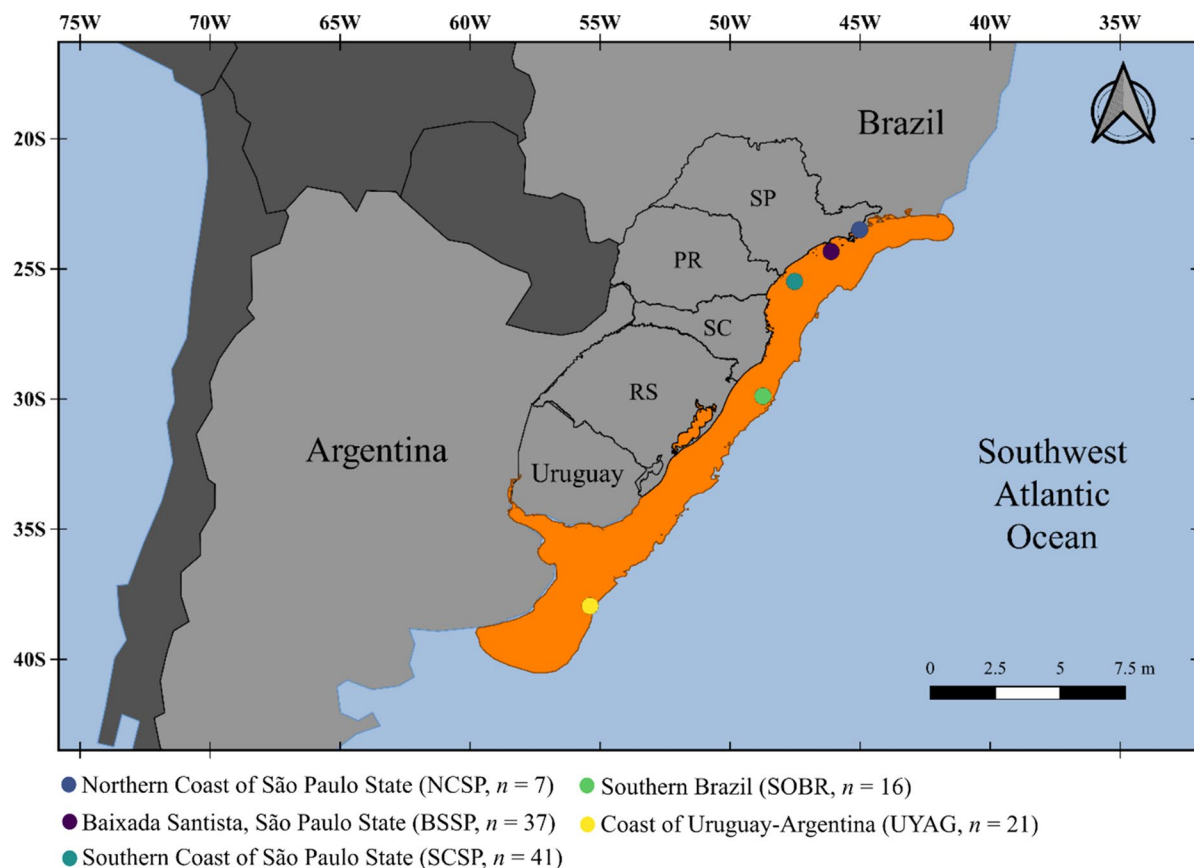


Fig. 1 Sampling of the angular angelshark (*Squatina guggenheim*) along the Southwest Atlantic Ocean. In orange, the species' geographic distribution (Oddone et al., 2019)

After amplification, samples were purified by a standard ExoSAP-IT™ cleanup protocol (Applied Biosystems™) and sequenced on an AB 3130 Genetic Analyzer (Applied Biosystems™).

DNA polymorphism and population genetic structure

The mtDNA sequences were aligned by the MUSCLE algorithm (Edgar, 2004) and edited in GENEIOUS 4.8.5 (Kearse et al., 2012). After the alignment, all mtDNA sequences were concatenated by SEQUENCEMATRIX 1.8 (Vaidya et al., 2011). The DNA polymorphism regarding the number of haplotypes (H), polymorphic sites (S), haplotype (h) and nucleotide (π) diversities were estimated in DNASP 6.12.03 (Rozas et al., 2017).

To test the null hypothesis of southwestern Atlantic panmixia, a population comparison for all sampling areas was estimated through the pairwise Φ_{ST} genetic differences using ARLEQUIN 3.5.2.2 (Excoffier

& Lischer, 2010) with the statistical significance tested by 50,000 permutations and significance level (α) = 0.05. The statistical significance of estimated probabilities was corrected using the Benjamini–Hochberg (1995) correction. A hierarchical framework of different hypothetical population scenarios was tested using an Analysis of Molecular Variance (AMOVA) (Arlequin 3.3.2.2; Excoffier et al., 1992). The scenarios were based on previous studies from Southwest Atlantic Ocean: (1) global population structure—panmictic population (Machado et al., 2020); (2) southeastern Brazil (NCSP, BSSP and SCSP) x southernmost regions (SOBR and UYAG) (Cruz et al., 2021); (3) Brazilian regions (NCSP, BSSP, SCSP and SOBR) x Uruguay-Argentina region (UYAG) (Marquéz et al., 2017); and (4) southeastern Brazil (NCSP, BSSP and SCSP) x southern Brazil (SOBR) x Uruguay-Argentina region (UYAG) (alternative scenario).

The number of historical migrants per generation ($Nm = \Theta.M$) among populations was inferred by MIGRATE-N 4.4.4 (Beerli & Felsenstein, 2001; Beerli & Palczewski, 2010). The software estimates, under a Bayesian approach, the mutation-scaled population sizes (Θ), defined as $\Theta = N_e\mu$ for mtDNA, and the mutation-scaled immigration rate (M), defined as $M = m/\mu$ where m is the immigration rate per generation and μ is the mutation rate per site and generation. Different migration hypotheses were tested, and the best model was assigned through the Bayes factor using Bezier scores from each run (Beerli & Palczewski 2010) (see Appendix 1 in Supplementary Material for details). The runs were performed with by an exponential prior distribution with fixed window ranging from Θ : 0–0.1 (Δ 0.01; 5000 bins) and M : 0–10,000,000 (Δ 1,000,000; 5000 bins). The MCMC settings were set as one long chain recorded by 200,000 steps incremented every 1000 steps with a single replicate comprising a total of 300,000,000 iterations. A static heating scheme of five chains with temperatures of 1.0, 1.33, 2.0, 4.0, 1,000,000 with a burn-in of 20% per chain. The convergence assignment was made in TRACER v1.7.1 (Rambaut et al., 2018).

Phylogeography and historical connectivity

Bayesian inference among haplotypes was constructed using BEAST v2.6.6 (Bouckaert et al., 2014) under an unlinked substitution model estimated for each locus using bModelTest (Bouckaert & Drummond, 2017). The tree was calibrated with fossil dating using the earliest Oligocene (33.17 ± 9.85 Mya) as the radiation and origin of the living extant Squatiniformes and an age of 12.18 ± 4.79 Mya, from the Middle Miocene, as the radiation of North and South America angel sharks (Stelbrink et al., 2010). The dated tree converged under a Birth–Death model (Stadler, 2010; Heath et al., 2014) and an uncorrelated lognormal relaxed clock (Drummond et al., 2005). The posterior distribution of parameters was estimated using the MCMC estimation, simulating three replications of 300,000,000 generations with 10% of burn-in. The assignment of convergence and the effective sample size (ESS) were performed in TRACER v1.7.2 (Rambaut et al., 2018). The software TREEANNOTATOR v2.6.6 (<http://beast.community/treannotator>) was used to summarize the trees into the

highest credibility tree. As outgroups, the sequences of *Squatina dumeril* Lesueur, 1818 (GenBank Access number MF993540) and *Squatina squatina* Linnaeus, 1758 (Genbank Access numbers KY464954 and MG029174). Support for nodes in the tree topology was obtained by posterior probability. The relationship among haplotypes and their geographic distribution was performed using a median-joining network as implemented in PopArt 1.7 (Leigh & Bryant, 2015).

Evidence for population expansion was evaluated by neutrality tests F_S (Fu, 1997) and R_2 (Ramos-Onsins & Rozas, 2002) with the statistical significance tested through 10,000 permutations by ARLEQUIN 3.5.2.2 and DNASP 6.12.03, respectively. Additionally, in order to reconstruct the past demography changes over time, an Extended Bayesian Skyline Plot (EBSP) was implemented in BEAST v2.6.2 (Bouckaert et al., 2014) for each population according to the most probable structure scenario since it must to be constructed over a panmictic assumption (Grant, 2015). The EBSP enables multiple unlinked loci in a single run, providing better estimates of demographic history over time under the stochastic coalescence process (Heled & Drummond, 2008). The evolutionary models for each locus of each population were estimated in jModelTest 2.1.10 (Darriba et al., 2012). A strict molecular clock was assigned due to its good approximation for analyses at the intrapopulation level and simplification of the coalescent model (Heled, 2010; Drummond & Bouckaert, 2015). The prior distributions followed the default suggestion, however, an MCMC of 300,000,000 generations was executed. Convergence and ESS (> 200) of the EBSP run were analyzed in TRACER v1.7.1 and the EBSP plot was visualized through plotEBSP.R script in R v3.6.1 (R Core Team, 2020; Heled & Drummond, 2008).

Results

DNA polymorphism and population genetic structure

The concatenated dataset CR-CytB-COI presented 2314 base pairs (bp) compounding 48 haplotypes, whereas CR (892 bp) (GenBank accession numbers MW456713-MW456735), CytB (792 bp) (GenBank® accession numbers MW456701-MW456712), and COI (630 bp) (GenBank® accession numbers

MW456691-MW456700) presented 23, 12 and 10 haplotypes, respectively (see Appendix 2 in Supplementary Material for details). Genetic diversity indices of each molecular marker dataset individually were described in the Supporting Information. The CR dataset presented the highest genetic diversity ($h_{CR} = 0.767 \pm 0.034$; $\pi_{CR} = 0.0023 \pm 0.0012$), whereas the lowest is showed in the CytB dataset ($h_{CytB} = 0.600 \pm 0.029$; $\pi_{CytB} = 0.0010 \pm 0.0026$). The concatenated dataset is characterized by high haplotype diversity ($h_{CR-CytB-COI} = 0.838 \pm 0.033$) and low nucleotide diversity ($\pi_{CR-CytB-COI} = 0.0017 \pm 0.0009$) (Table 1). At the population-level, diversity indices were lower within southeastern Brazil regions (Baixada Santista, São Paulo State – BSSP and Southern Coast of São Paulo State—SCSP) (Table 1). In general, both concatenated and single molecular markers showed, for some populations, similar patterns of haplotype and nucleotide diversities (see Appendix 2 in Supplementary Material for details).

The global population structure reveals the presence of high and significant population genetic structure in *S. guggenheim* ($\Phi_{ST} = 0.2278$, $p < 0.01$), failing to accept the null hypothesis of panmixia for *S. guggenheim* along the sampled region in the Southwest Atlantic Ocean. The Φ_{ST} pairwise showed significant population structure among all sampling regions, except for NCSP (Table 2). In particular, the genetic differentiation showed by the pairwise Φ_{ST} analysis is greater between southeastern Brazil and southernmost regions; however, high genetic population structure can be observed even between closely sampling regions (BSSP and SCSP: $\Phi_{ST} = 0.1695$, p

Table 2 Pairwise Φ_{ST} estimates for sampled regions of *Squatina guggenheim* based on the concatenated sequence dataset [control region (CR)–cytochrome *b* (CytB)–cytochrome *c* oxidase I (COI): CR–CytB–COI]

	NCSP	BSSP	SCSP	SOBR	UYAG
NCSP	–	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	0.002
BSSP	0.0922	–	0.002	0.0477	0.002
SCSP	0.0497	0.1695	–	0.002	0.002
SOBR	0.0875	0.0710	0.2617	–	0.0463
UYAG	0.3180	0.2475	0.4927	0.0476	–

Bold font indicates significant values after Benjamini–Hochberg correction ($p < 0.05$). *n.s.* non-significative values. NCSP Northern Coast of São Paulo State, BSSP Baixada Santista, São Paulo State, SCSP Southern Coast of São Paulo State, SOBR Southern Brazil, UYAG Coast of Uruguay-Argentina

< 0.02). On the other hand, the hierarchical AMOVA showed no statistically significant population structure for any of the tested scenarios (Table 3), which might be associated with the presence of migrants among populations. Despite that, following the pairwise Φ_{ST} evidence we suggest the presence of at least four populations: population 1: Northern Coast of São Paulo (NCSP) and Baixada Santista (BSSP)—NBSP; population 2: Southern Coast of São Paulo (SCSP)—SCSP; population 3: southern Brazil (SOBR)—SOBR; population 4: Coast of Uruguay-Argentina (UYAG)—UYAG. The Φ_{ST} pairwise and AMOVA results for each molecular marker are displayed in Supplementary Information (Appendix 2).

Regarding the historical gene flow, the best migration model (see Appendix 2 in Supplementary Material for details) displayed a stepping-stone dispersal

Table 1 Summary statistics of sequence variability and population demographic parameters of *Squatina guggenheim* for the concatenated sequence dataset in each sampling region [con-

Region	<i>n</i>	<i>H</i>	<i>S</i>	<i>h</i> ± <i>sd</i>	π ± <i>sd</i>	<i>F_S</i>	<i>R₂</i>
NCSP	7	6	6	0.952 ± 0.096	0.0011 ± 0.0010	– 2.70811	0.2267
BSSP	37	10	8	0.818 ± 0.048	0.0015 ± 0.0008	– 0.43468	0.1158
SCSP	41	11	9	0.502 ± 0.096	0.0008 ± 0.0009	– 3.4509	0.1165
SOBR	16	13	23	0.967 ± 0.036	0.0024 ± 0.0030	– 5.1776	0.1438
UYAG	21	17	23	0.976 ± 0.023	0.0017 ± 0.0027	– 11.4717	0.1353
Overall	122	48	34	0.838 ± 0.033	0.0017 ± 0.0009	– 4.6486	0.0896

n, sample size; *H*, number of haplotypes; *S*, polymorphic sites; *h*, haplotype diversity; π , nucleotide diversity; *F_S* Fu's (1996) and *R₂* (Ramos-Onsins & Rozas, 2002) neutrality tests. Bold font indicates significant values ($p < 0.05$ for *R₂* and $p < 0.02$ for *F_S*) after Benjamini–Hochberg correction. NCSP Northern Coast of São Paulo State, BSSP Baixada Santista, São Paulo State, SCSP Southern Coast of São Paulo State, SOBR Southern Brazil, UYAG Coast of Uruguay-Argentina

Table 3 Analysis of Molecular Variance (AMOVA) for hypothetical genetic structure scenarios of *Squatina guggenheim* based on the concatenated sequence dataset [control region(CR)—cytochrome *b* (CytB)—cytochrome *c* oxidase I (COI): CR—CytB—COI] with hierarchical genetic structuring scenarios among sampled regions

Scenario	Hypothetical structuring scenarios	Source of variation	%V	Fixation indexes	<i>p</i> value
1	(NCSP + BSSP + SCSP + SOBR + UYAG)	Among populations	77.21	$\Phi_{ST} = \mathbf{0.22785}$	< 0.0001
		Within populations	22.79		
2	(NCSP + BSSP + SCSP) × (SOBR + UYAG)	Among groups	21.36	$\Phi_{CT} = 0.21359$	0.11243
		Among populations within groups	8.56	$\Phi_{SC} = 0.10888$	0.00833
		Within populations	70.08	$\Phi_{ST} = \mathbf{0.29922}$	< 0.0001
3	(NCSP + BSSP + SCSP + SOBR) × (UYAG)	Among groups	18.69	$\Phi_{CT} = 0.22682$	0.19978
		Among populations within groups	9.47	$\Phi_{SC} = \mathbf{0.14119}$	< 0.0001
		Within populations	71.84	$\Phi_{ST} = \mathbf{0.33599}$	< 0.0001
4	(NCSP + BSSP) × (SCSP) × (SOBR) × (UYAG)	Among groups	15.25	$\Phi_{CT} = 0.15251$	0.40376
		Among populations within groups	7.79	$\Phi_{SC} = 0.09189$	0.11243
		Within populations	76.96	$\Phi_{ST} = \mathbf{0.23039}$	< 0.0001

Fixation indexes defined as Φ_{ST} , permutation of haplotypes among populations among groups; Φ_{SC} , permutation of haplotypes among populations within groups; Φ_{CT} , permutation of populations among groups. %V, Percentage of variation, *p* value, the marginal significance within a statistical hypothesis test representing the probability of the occurrence of a given event. Sampling regions: NCSP Northern Coast of São Paulo State, BSSP Baixada Santista, São Paulo State, SCSP Southern Coast of São Paulo State, SOBR Southern Brazil, UYAG Coast of Uruguay-Argentina. In bold, significant values after Benjamin-Hochberg correction ($p < 0.005$)

evidenced by an intense and asymmetric number of migrants per generation southward along the Southwest Atlantic coastline (Fig. 2). The mutation-scaled population sizes (Θ) resulted in symmetric mutation-scaled population size (Θ) of $\Theta_{CR+CytB+COI} = 0.00217$ (0.00122–0.00308 95% HPD) for each population. Bayesian estimates for each molecular marker, including the values of mode, median, mean, and percentiles of the posterior probability density for each parameter are displayed in Supplementary Material (Appendix 2).

Phylogeography and historical connectivity

The main clades within *S. guggenheim* revealed by the Bayesian inference were not statistically significant according to posterior probability values; therefore, the dichotomy founded in the phylogeny cannot be used to support any historical event (Supplementary Material Appendix 2—Fig. 3). The haplotype network presented an overall star shape and most haplotypes are separated by only a few mutational steps (Fig. 3). The haplotype H3 ($n = 48$) shows the historical connectivity among all sampling regions, except UYAG (Fig. 3). The haplotype network of each molecular marker is available in the Supplementary Information (Appendix 2—Fig. 1).

We performed all the demography tests under the genetic structure of 4 populations, following the results of the pairwise Φ_{ST} . Signs of past population expansion were revealed by both neutrality tests; however, only R_2 test was statistically significant for all regions (Table 1). The EBSP reconstruction showed signs of population expansion for SOBR and UYAG populations, while revealed a flat curve for NBSP and SCSP populations (Fig. 4). The SOBR population presented a constant smooth population expansion with a start point about 50 thousand years ago (Kya) with no demographic alterations during LGM until the present days, while UYAG population showed a marked population expansion after LGM at about 10 Kya, in line with the first period of deglaciation (Fig. 4c and d).

Discussion

Nowadays, population genetics studies have shown essential information for the elaboration of effectively management and conservation strategies for many endangered sharks and rays (Dudgeon et al., 2012; Domingues et al., 2018). Here we provide the population genetic and phylogeographic assessment of the angular angelshark (*Squatina guggenheim*) for

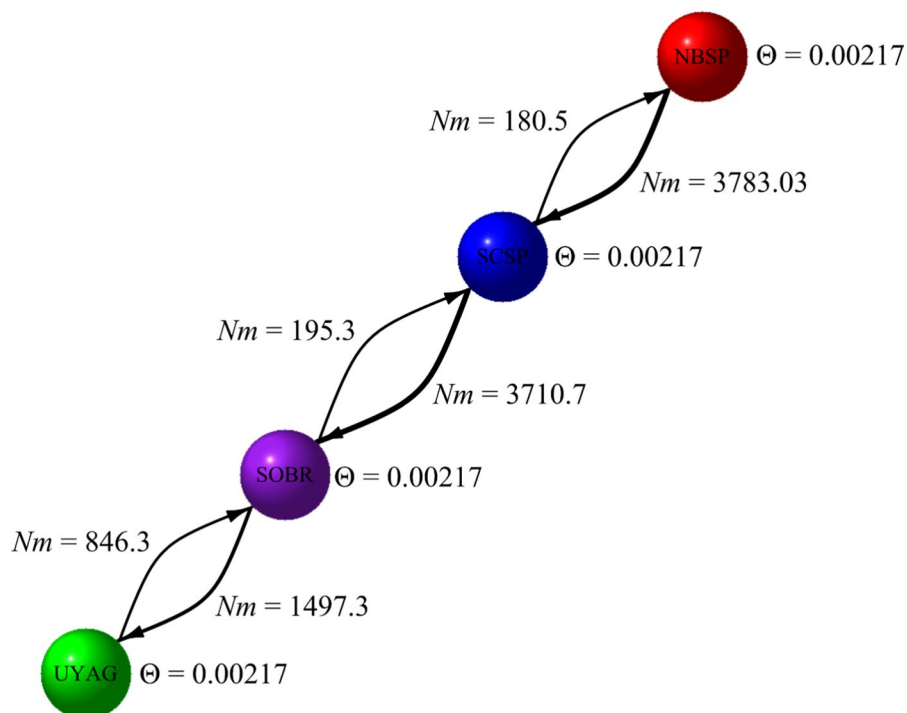


Fig. 2 Number of historical migrants per generation of *Squatina guggenheim* in the Southwest Atlantic Ocean based on the concatenated sequence dataset [control region (CR)–cytochrome *b* (CytB)–cytochrome *c* oxidase I (COI): CR–CytB–COI]. The values indicate the estimations for the number of migrants per generation ($Nm = \Theta.M$) where Θ is the

mutation-scaled population size and M , the mutation-scaled immigration rates. This analysis was performed among four populations according to Φ_{ST} analysis. Populations: *NBSP* Northern Coast of São Paulo (NCSP) and Baixada Santista São Paulo State (BSSP), *SCSP* Southern Coast of São Paulo State, *SOBR* Southern Brazil, *UYAG* Coast of Uruguay-Argentina

its main distribution in the Southwest Atlantic Ocean, based on a multilocus mitochondrial DNA approach. Significant population genetic structure was detected among Southwest Atlantic regions, due to behavior of angel sharks and oceanographic particularities of the Southwest Atlantic Ocean. Also, the evolutionary history of *S. guggenheim* revealed signs of population expansion for southern Southwest Atlantic populations during the Pleistocene.

DNA polymorphism and population genetic structure

The CR–CytB–COI concatenated dataset revealed high haplotype diversity and low nucleotide diversity for *S. guggenheim* in the Southwest Atlantic. Analogous values of haplotype diversity were found in elasmobranchs with similar biological and ecological traits (e.g., *Squatina californica* Ayres, 1859—Ramírez-Amaro et al., 2017; *Raja clavata* Linnaeus, 1758—Chevolot et al., 2006; *Pseudobatos productus*

Ayres, 1854—Sandoval-Castillo et al., 2004). The low nucleotide diversity found in *S. guggenheim* may be related to changes in the coastal habitats due to past climate fluctuations in the Southwest Atlantic (Grant & Bowen, 1998; O'Brien et al., 2013). In addition, low nucleotide diversity is present in many species that are under intense fishing pressure (Domingues et al., 2018). Low genetic diversity is considered a common pattern in the elasmobranch group due to its slow rates of molecular evolution and life history characteristics (O'Brien et al., 2013; Domingues et al., 2018). In contrast, this genetic diversity pattern (high haplotype diversity and low nucleotide diversity) is not consistent with the previous regional study by García et al. (2014), which can be justified by its narrow geographic sampling scale.

Substantial intraspecific differences were observed among populations, where southernmost regions (SOBR and UYAG) showed higher levels of genetic diversity and a larger number of unique haplotypes

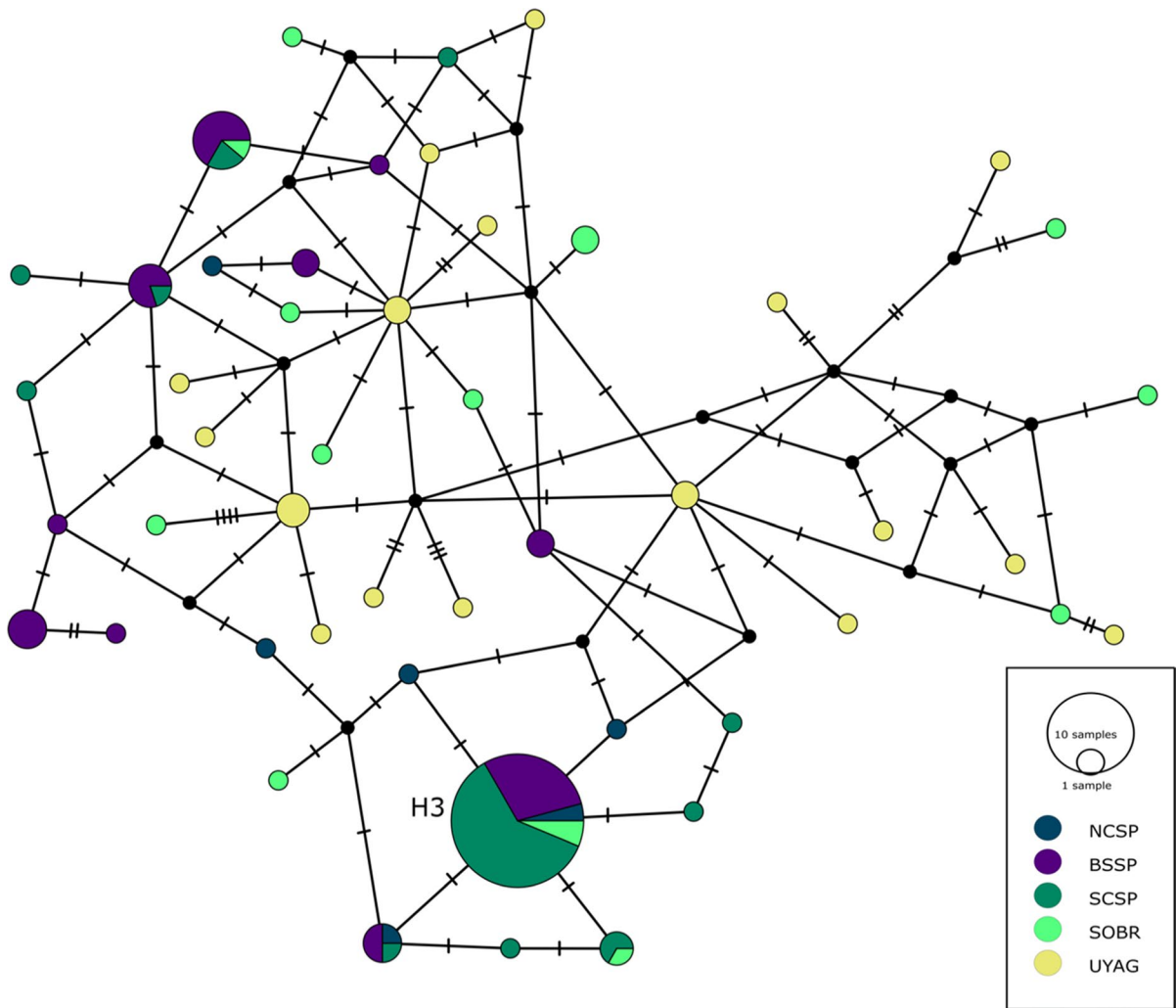


Fig. 3 Median-joining haplotype network of *Squatina guggenheim* in the Southwest Atlantic Ocean based on the concatenated sequence dataset [control region (CR)–cytochrome *b* (CytB)–cytochrome *c* oxidase I (COI): CR–CytB–COI]. H3 refers to the most frequent haplotype. Color codes represent sampling regions: *NCSP* Northern Coast of São Paulo State,

BSSP Baixada Santista, São Paulo State, *SCSP* Southern Coast of São Paulo State; *SOBR*: Southern Brazil, *UYAG* Coast of Uruguay–Argentina. The circle size is proportional to the frequency of each haplotype. The segments represent one mutation event, and the black dots represent hypothetical haplotypes not sampled

compared to southeastern Brazil regions (BSSP and SCSP), as well as reported for the Brazilian sharpnose shark, *Rhizoprionodon lalandii* Müller & Henle, 1839, in the same region (Mendonça et al., 2013). EBSP reinforces this pattern with the evidence of demographic expansion in both populations, suggesting that populations from the southern Southwest Atlantic underwent a rapid population growth after habitat restriction during glacial periods (Grant & Bowen, 1998). In addition, the high levels of primary

productivity due to freshwater discharges (Patos Lagoon—32°S and Rio de La Plata—35°S) and the hypothesis of this region being the birth and nursery areas of *S. guggenheim* may have been contributing to the high genetic diversity observed (Viñas et al., 2002; Vögler et al., 2008). Even though García et al. (2014) had reported genetic structuring only between coastal and outer-shelf regions (longitudinal population structure), all analyses herein showed high latitudinal genetic population structure among

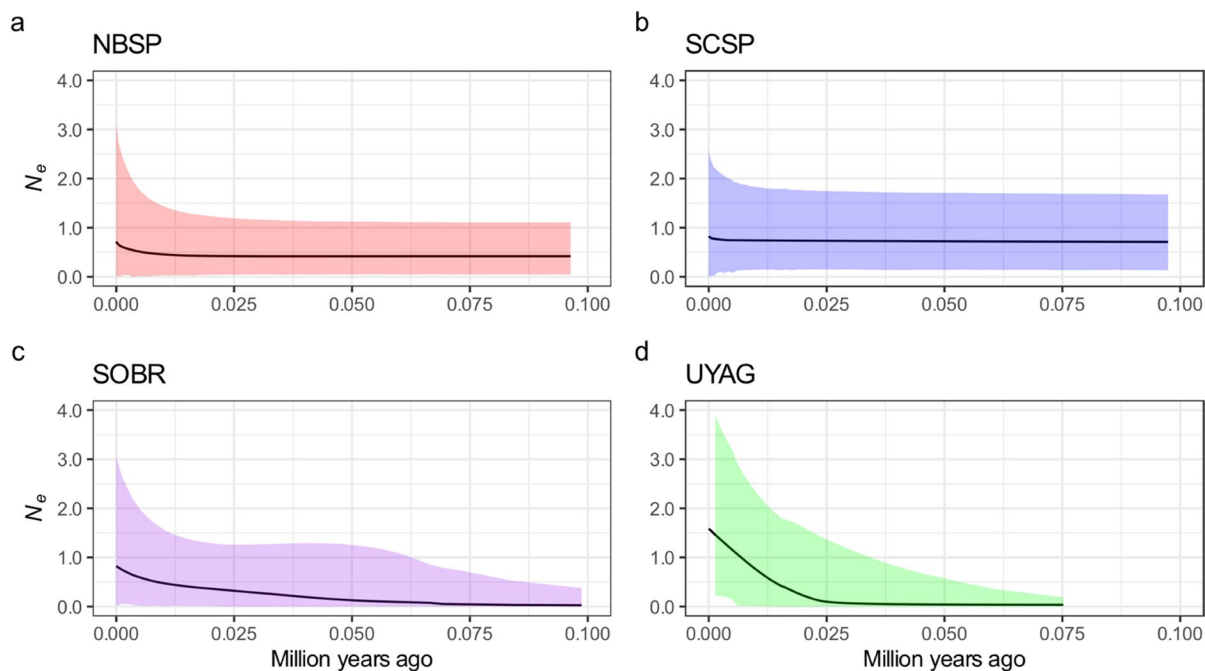


Fig. 4 Extended Bayesian Skyline Plots (EBSPs) for *Squatina guggenheim* in the Southwest Atlantic Ocean based on the concatenated sequence dataset [control region (CR)–cytochrome *b* (CytB)–cytochrome *c* oxidase I (COI): CR–CytB–COI]. The *x*-axis is in units of millions of years ago (Mya), and the *y*-axis is the effective population size (N_e). The mean and 95% highest posterior density (HPD) intervals are calculated for the population sizes for the period leading to an estimated plot of popu-

lation size through time with an associated measure of uncertainty. This analysis was performed among four populations according to the pairwise Φ_{ST} analysis. All four populations are shown: **a** NBSP—Northern Coast of São Paulo (NCSP) and Baixada Santista, São Paulo State (BSSP); **b** SCSP—Southern Coast of São Paulo State; **c** SOBR—Southern Brazil; **d** UYAG—Coast of Uruguay-Argentina

S. guggenheim populations in the Southwest Atlantic Ocean. Given the strong and statistically significant results of Φ_{ST} pairwise estimations, we suggest the presence of at least four populations. However, we detected the presence of gene flow among populations as showed by number of historical migrants per generation, which might explain the absence of significant structure for the scenarios tested in the AMOVA analysis.

Although in a larger scale, Rodrigues et al. (2014) suggested the influence of the oceanographic particularities of the Southwest Atlantic Ocean in the population structure of *Macrodon atricauda* Günther, 1880, where the subtropical shelf front near the Patos Lagoon (33°S) divides the Southwest Atlantic shelf into two regions: warm and salty waters (northern region), and cold and less salty waters (southern regions). These notable environmental variations may also be responsible for the population structure of *S. guggenheim* since greater genetic differentiations

occur between southeastern Brazil populations and southernmost populations. In addition, this hypothesis may bring interesting historic assumptions about the influence of glacial cycles in the *S. guggenheim* population, as well as bring up important insights about climatic variations of current times, since environmental variables are responsible to modulate feeding process and reproductive behaviors, affecting dispersal and connectivity among populations in the Southwest Atlantic (Miranda & Haimovici, 2007; Vaz-dos-Santos et al., 2009; Vasconcellos et al., 2015).

Similarly, the Pacific angelshark *S. californica* displays regional genetic differentiation between the Pacific Ocean and the Gulf of California (Ramírez-Amaro et al., 2017). The genetic heterogeneity within the genus *Squatina* reflects their biological characteristics, such as low dispersal ability and preference for shallow-coastal habitats, resulting in lower migration movements between nearby geographical areas

(Gaida, 1997; Vooren & Klippel, 2005; Colonello et al., 2007). On another hypothesis, the reproductive behavior may contribute to genetic divergence among populations, as reported in other coastal elasmobranchs (Chapman et al., 2015). Many species of angel sharks exhibit seasonal inshore-offshore migrations, wherein females moving inshore to give birth, including *S. guggenheim* (Colonello et al., 2007; Vögler et al., 2008; Ellis et al., 2020). However, no satellite tagging nor genetic studies had determined the presence of philopatric behavior in angel sharks. In addition, differences in life history parameters such as maximum length and length at-maturity were previously reported for *S. guggenheim* in the Southwest Atlantic, suggesting the existence of different stocks (Colonello et al., 2007; Ellis et al., 2020). In a recent study, Cruz et al. (2021) showed that the levels of genetic differentiation in guitarfishes along the Southwest Atlantic Ocean might be explained by the resident behavior of the species, which only perform depth migrations during the reproductive cycle. Heterogeneous populations are commonly observed within coastal sedentary elasmobranchs (e.g., *Neotrygon kuhlii* Müller & Henle, 1841—Borsa et al., 2012; *Ginglymostoma cirratum* Bonnaterre, 1788—Karl et al., 2012). These biological characteristics contribute to form discrete local or regional populations, leading a high susceptibility to overexploitation in short term (Domingues et al., 2018).

Despite the high levels of population differentiation, the Bayesian analysis of historical gene flow revealed strong stepping-stone connectivity among Southwest Atlantic populations, following especially the Brazil Current. The Brazil Current (BC) presented accentuated climatic variations in the Late Pleistocene (126 ka–11.7 ka) during the Marine Isotope Stage (MIS) 5, with a warm temperature peak (> 25°C) and the highest surface water salinity in the interglaciation substage 5e (Santos et al., 2017). Those environmental variations may have impacted some individuals, suggesting the existence of migration from southeastern Brazil toward the Brazil-Malvinas/Falkland Confluence region (38°S), which present less saline (34.6–36) and colder (~ 6–20°C) waters (Vögler et al., 2008; Santos et al., 2017). Similarly, this pattern was currently observed on the southeastern Brazilian coast, in which climatic variations of the past four decades had led coastal fishes to move southward looking for more favorable

environments (Araújo et al., 2018). However, the stepping-stone pattern of gene flow is in line with the Φ_{ST} pairwise estimations, suggesting the absence of connectivity between southeastern Brazil (NBSP and SCSP) and UYAG populations.

Even though the lowest number of migrants occur between regions that are separated by the Rio de La Plata discharges (SOBR and UYAG populations), our sequences from Uruguay, previously included in the UYAG population for the analyses, demonstrate a higher genetic similarity to Argentine individuals than to ones of southern Brazil (SOBR). This pattern suggests that individuals living around the Rio de La Plata are equally adapted to the environmental particularities of the region. In addition, a possible explanation to the accentuated connectivity between Uruguay and Argentine individuals is the high levels of primary productivity around the Rio de La Plata's discharge, being considered as an important feeding region. Furthermore, it is a place of massive spawning of the *Engraulis anchoita* Hubbs & Marini, 1935, the fish most consumed by *S. guggenheim*. In this sense, this region may be considered an area of the confluence of populations (Viñas et al., 2002; Vögler et al., 2008). A similar pattern was observed for *Rhizoprionodon porosus* Poey, 1861 in the region of Amazon River discharges (Mendonça et al., 2011). Otherwise, northern populations (SOBR, SCSP, NBSP) did not present this connectivity with UYAG population, showing that freshwater discharges of the Rio de La Plata work as a biogeographic barrier for *S. guggenheim*.

Phylogeography and historical demography

Since the clades within *S. guggenheim* did not present enough statistical support to be considered divergent lineages, it suggests that even during sea surface cooling periods' as well as variation in sea levels during the Miocene/Pliocene the populations of *S. guggenheim* were still able to sustain gene flow. As a result, the rise of divergent lineages was not observed on the molecular markers used in this study; therefore, only the genetic population structure is responsible to explain the differences between the geographical regions. (Herbert et al., 2016; Toomey et al., 2016). Even though the haplotype network had shown a star-like shape, the most common haplotype H3 ($n = 48$) highlights a gradual historic divergence between

southeastern Brazil and southernmost regions due to only 6.25% of its composition being consisted of individuals from SOBR region. Moreover, H3 is not present in the UYAG region.

Signs of population expansion were observed in *S. guggenheim* for all populations based on R_2 results (Ramos-Onsins & Rozas, 2002); however, for F_s (Fu, 1997) only UYAG population is statistical significantly, which is consistent with its genetic diversity indices (high haplotype and low nucleotide diversity), and with the presence of exclusive haplotypes (Grant & Bowen, 1998). Sudden population expansion is often likely to occur after episodes of glacial cycles, such as occurred for many species during the Pleistocene period (Grant, 2015; O'Brien et al., 2013). The Pleistocene was marked by intense climatic and sea-level oscillations, several habitats along the Southwest Atlantic Ocean were considered unsuitable for coastal species due to sea-level declines, especially during the LGM, when the relative sea-level was approximately 130 m below the present level (Corrêa, 1996; Ludt & Rocha, 2015; Alves & Mahiques, 2019), affecting the population dynamics of many coastal marine fishes in this region (Rabassa et al., 2005; Santos et al., 2006; Iriarte et al., 2011; O'Brien et al., 2013; Machado et al., 2017). Even though the EBSP analysis suggests events of population expansion for SOBR and UYAG populations, matching with neutrality tests' findings, these results could lead to overinterpretation of the population history of *S. guggenheim*. Both populations present a higher number of unique haplotypes, which might have overestimated values of population size. Additionally, the flat curve showed by the EBSP reconstruction, especially for NBSP and SCSP populations, is not an indication of population stability, but may be related to the extinction of haplotype lineages due to population contractions during glacial periods (Grant, 2015). Some weakness of the present study supports this conclusion, such as small sample sizes of each population, gene flow among populations, genetic divergence within populations and molecular clock calibration (Grant, 2015).

Conclusion

The present study suggests the existence of population genetic structure in *Squatina guggenheim*. This

heterogeneity may be addressed to its biological characteristics, such as limited dispersal, preference for shallow waters and to reproductive behaviors (Ellis et al., 2020). Moreover, given the uniqueness of Southwest Atlantic Ocean, we suggest that environmental features also have an important influence in modulating *S. guggenheim* dispersal, especially salinity and thermal variations, though it should be better investigated since this influence was previously reported only during reproductive periods (Vögler et al., 2008).

In general, *S. guggenheim* presents moderate levels of genetic diversity, which may prejudice its evolutionary potential in long-term (Grant & Bowen, 1998; Domingues et al., 2018). In addition, the region with the lowest genetic diversity indices (SCSP) faces a lack of official annual reports on landings for *S. guggenheim* monitoring (ICMBio/MMA, 2018). Despite the high genetic diversity observed in the SOBR population, current fishing data showed high rates of overfishing for this region, especially in the 1990s, after the implantation of fisheries targeting angel sharks through bottom gillnets (Vooren & Klippel, 2005; Ellis et al., 2020), which may impact its long-term evolutionary process (Belgrano & Fowlet, 2013; Domingues et al., 2018).

Given those particularities, the genetic heterogeneity among *S. guggenheim* populations must be considered in official landing reports and in the development of conservation strategies to preserve its adaptive potential. Since this species is classified as "Endangered" by the IUCN Red List with signs of population decreasing, conservation efforts must be coordinate among countries in an international collaboration framework to implement efficient strategies at both local and regional scales. Although some conservation actions are currently being carried out for *S. guggenheim* (Oddone et al., 2019), there are still recent signs of landings, mainly caught through bycatch (Almerón-Souza et al., 2018; Bunholi et al., 2018). Hence, the identification of genetic population and phylogeographic patterns through the present study will contribute to better understand the dynamic of this endemic and endangered species in the Southwest Atlantic, as well as support the elaboration of international management and conservation strategies in the region.

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Author contribution IVB, RRD and FFM conceived the ideas. MMR, JMC, MG, SG, RHAF provided sampling. IVB carried out laboratory work. IVB and BLSF analyzed the data. IVB led the writing of the first draft. All authors contributed to manuscript revision.

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Data availability DNA sequences of all molecular markers have been submitted to GenBank (<https://www.ncbi.nlm.nih.gov/genbank>), accession numbers: MW456691- MW456735.

Code availability Not applicable.

Declarations

Conflict of interest The authors have no conflict of interests.

Ethics approval Not applicable.

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Consent for publication Not applicable.

References

- Acha, E. M., H. W. Mianzan, R. A. Guerrero, M. Favero & J. Bava, 2004. Marine fronts at the continental shelves of austral South America: physical and ecological processes. *Journal of Marine Systems* 44: 83–105.
- Allendorf, F. W., G. Luikart & S. N. Aitken, 2013. Conservation and the genetics of population, Blackwell, Oxford:
- Almerón-Souza, F., C. Sperb, C. L. Castilho, P. I. Figueiredo, L. T. Goncalves, R. Machado, et al., 2018. Molecular identification of shark meat from local markets in Southern Brazil based on DNA barcoding: Evidence for mislabeling and trade of Endangered species. *Frontiers in Genetics*. <https://doi.org/10.3389/fgene.2018.00138>.
- Alves, D. P. & M. M. de Mahiques, 2019. Deposition and sea-level evolution models for Upper Pleistocene/Holocene in the São Sebastião Channel (SE Brazilian coast) inferred from 5th order seismic stratigraphy. *Journal of South American Earth Sciences* 93: 382–393.
- Araújo, F. G., T. P. Teixeira, A. P. P. Guedes, M. C. C. de Azevedo & A. L. M. Pessanha, 2018. Shifts in the abundance and distribution of shallow water fish fauna on the southeastern Brazilian coast: a response to climate change. *Hydrobiologia* 814: 205–218.
- Avise, J. C., 2000. *Phylogeography—the history and formation of species*, Harvard University Press:
- Avise, J. C., 2009. *Phylogeography: retrospect and prospect*. *Journal of Biogeography* 36: 3–15.
- Beerli, P. & J. Felsenstein, 2001. Maximum likelihood estimation of a migration matrix and effective population sizes in n subpopulations by using a coalescent approach. *Proceedings of the National Academy of Sciences* 98: 4563–4568.
- Beerli, P. & M. Palczewski, 2010. Unified framework to evaluate panmixia and migration direction among multiple sampling locations. *Genetics* 185: 313–326.
- Beheregaray, L. B., P. Sunnucks & D. A. Briscoe, 2002. A rapid fish radiation associated with the last sea-level changes in southern Brazil: the silverside *Odontesthes perugiae* complex. *Proceedings of the Royal Society of London Series B: Biological Sciences* 269: 65–73.
- Belgrano, A. & C. W. Fowler, 2013. How fisheries affect evolution. *Science* 342: 1176–1177.
- Benjamini, Y. & Y. Hochberg, 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society. Series B (methodological)* 57: 289–300.
- Borsa, P., I. S. Arlyza, M. Laporte & P. Berrebi, 2012. Population genetic structure of blue-spotted maskray *Neotrygon kuhlii* and two other Indo-West Pacific stingray species (Myliobatiformes: Dasyatidae), inferred from size-polymorphic intron markers. *Journal of Experimental Marine Biology and Ecology* 438: 32–40.
- Bouckaert, R., J. Heled, D. Kühnert, T. Vaughan, C. H. Wu, D. Xie, et al., 2014. BEAST 2: a software platform for Bayesian evolutionary analysis. *PLoS Computational Biology* 10: e1003537.
- Bouckaert, R. R. & A. J. Drummond, 2017. bModelTest: Bayesian phylogenetic site model averaging and model comparison. *BMC Evolutionary Biology* 17(1): 1–11.
- Bowen, B. W., K. Shanker, N. Yasuda, M. Celia, M. C. M. D. Malay, S. von der Heyden, et al., 2014. Phylogeography unplugged: comparative surveys in the genomic era. *Bulletin of Marine Science* 90: 13–46.
- Bunholi, I. V., B. L. S. Ferrette, J. B. De Biasi, C. O. Magalhães, M. M. Rotundo, C. Oliveira, et al., 2018. The fishing and illegal trade of the angelshark: DNA barcoding against misleading identifications. *Fisheries Research* 206: 193–197.
- Chapman, D. D., K. A. Feldheim, Y. P. Papastamatiou & R. E. Hueter, 2015. There and back again: A review of residency and return migration in sharks, with implication for populations structure and management. *Annual Review of Marine Science* 7: 547–570.

- Chevolut, M., G. Hoarau, A. D. Rijnsdorp, W. T. Stam & J. L. Olsen, 2006. Phylogeography and population structure of thornback rays (*Raja clavata* L., Rajidae). *Molecular Ecology* 15: 3693–3705.
- Chiaromonte, G. E., 1998. Shark fisheries in Argentina. *Marine and Freshwater Research* 49: 601–609.
- Colonello, J. H., L. O. Lucifora & A. M. Massa, 2007. Reproduction of the angular angelshark (*Squatina guggenheim*): geographic differences, reproductive cycle, and sexual dimorphism. *ICES Journal of Marine Science* 64: 131–140.
- Corrêa, I. C. S., 1996. Les variations du niveau de la mer durant les derniers 17.500 ans BP: l'exemple de la plateforme continentale du Rio Grande do Sul-Brésil. *Marine Geology* 130: 163–178.
- Cortinhas, M. C. S., R. Kersanach, M. Proietti, L. F. C. Dumont, F. D'Incao, A. L. F. Lacerda & M. M. Cesari, 2016. Genetic structuring among silverside fish (*Atherinella brasiliensis*) populations from different Brazilian regions. *Estuarine, Coastal and Shelf Science* 178: 148–157.
- Cousseau, M. B. & D. E. Figueroa, 2001. Las especies del género *Squatina* en aguas de Argentina (Pisces: Elasmobranchii: Squatinidae). *Neotrópica* 47: 85–86.
- Cruz, V. P., A. M. Adachi, P. H. Oliveira, G. S. Ribeiro, F. G. Paim, B. C. Souza, et al., 2021. Genetic diversity in two threatened species of guitarfish (Elasmobranchii: Rhinobatidae) from the Brazilian and Argentinian coasts: an alert for conservation. *Neotropical Ichthyology*. <https://doi.org/10.1590/1982-0224-2021-0012>.
- Cuevas, J. M., V. García, S. Montealegre-Quijano, L. Paesch, M. Estalles, V. Falabella, et al., 2020. Report of the IUCN Regional Red List Workshop for Species of the Patagonian Sea: Chondrichthyans. *Forum for the Conservation of the Patagonian Sea*. 302 pp.
- Darriba, D., G. L. Taboada, R. Doallo & D. Posada, 2012. jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* 9: 772–772.
- DiBattista, J. D., C. Wilcox, M. T. Craig, L. A. Rocha & B. W. Bowen, 2011. Phylogeography of the Pacific Blue-line Surgeonfish, *Acanthurus nigroris*, reveals high genetic connectivity and a cryptic endemic species in the Hawaiian Archipelago. *Journal of Marine Biology* 2011: 1–17.
- Domingues, R. R., A. W. S. Hilsdorf & O. B. F. Gadig, 2018. The importance of considering genetic diversity in shark and ray conservation policies. *Conservation Genetics* 19: 501–525.
- Drummond, A. J., A. Rambaut, B. Shapiro & O. G. Pybus, 2005. Bayesian coalescent inference of past population dynamics from molecular sequences. *Molecular Biology and Evolution* 22: 1185–1192.
- Dudgeon, C. L., D. C. Blower, D. Broderick, J. L. Giles, B. J. Holmes, T. Kashiwagi, et al., 2012. A review of the application of molecular genetics for fisheries management and conservation of sharks and rays. *Journal of Fish Biology* 80: 1789–1843.
- Dulvy, N. K., J. K. Baum, S. Clarke, L. J. Compagno, E. Cortés, A. Domingo, et al., 2008. You can swim but you can't hide: the global status and conservation of oceanic pelagic sharks and rays. *Aquatic Conservation* 18: 459–482.
- Drummond, A. J., & Bouckaert, R. R. (2015). *Bayesian evolutionary analysis with BEAST*. Cambridge University Press.
- Dulvy, N. K., S. L. Fowler, J. A. Musick, R. D. Cavanagh, P. M. Kyne, L. R. Harrison, et al., 2014. Extinction risk and conservation of the world's sharks and rays. *eLife* 3: e00590.
- Edgar, R. C., 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* 32: 1792–1797.
- Ebert, D. A., S. L. Fowler & L. J. Compagno, 2013. *Sharks of the world: a fully illustrated guide*, Wild Nature Press, Plymouth:
- Ellis, J. R., J. Barker, S. R. M. Phillips, E. K. Meyers & M. Heupel, 2020. Angel sharks (Squatinidae): a review of biological knowledge and exploitation. *Journal of Fish Biology*. <https://doi.org/10.1111/jfb.14613>.
- Excoffier, L. & H. E. Lischer, 2010. Arlequin suite version 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources* 10: 564–567.
- Excoffier, L., P. E. Smouse & J. M. Quattro, 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics* 131: 479–491.
- Felsenstein, J., 1982. How can we infer geography and history from gene frequencies? *Journal of Theoretical Biology* 96: 9–20.
- Franco, B. C., O. Defeo, A. R. Piola, M. Barreiro, H. Yang, L. Ortega & O. O. Moller, 2020. Climate change impacts on the atmospheric circulation, ocean, and fisheries in the southwest South Atlantic Ocean: a review. *Climatic Change* 162: 2359–2377.
- Fruet, P. F., E. R. Secchi, J. C. Di Tullio, P. C. Simões-Lopes, F. Daura-Jorge, A. P. Costa, et al., 2017. Genetic divergence between two phenotypically distinct bottlenose dolphin ecotypes suggests separate evolutionary trajectories. *Ecology and Evolution* 7(21): 9131–9143.
- Fu, Y. X., 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics* 147: 915–925.
- Gaida, I. H., 1997. Population structure of the Pacific angelshark, *Squatina californica* (Squatiniformes: Squatinidae), around the California Channel Islands. *Copeia* 1997: 738–744.
- García, G., S. Pereyra, V. Gutierrez, S. Oviedo, P. Miller & A. Domingo, 2014. Population structure of *Squatina guggenheim* (Squatiniformes, Squatinidae) from the south-wester Atlantic Ocean. *Journal of Fish Biology* 86: 186–202.
- Grant, W. A. S. & B. W. Bowen, 1998. Shallow population histories in deep evolutionary lineages of marine fishes: insights from sardines and anchovies and lessons for conservation. *Journal of Heredity* 89: 415–426.
- Grant, W. S., 2015. Problems and cautions with sequence mismatch analysis and Bayesian skyline plots to infer historical demography. *Journal of Heredity* 106: 333–346.
- Heath, T. A., J. P. Huelsenbeck & T. Stadler, 2014. The fossilized birth–death process for coherent calibration of divergence-time estimates. *Proceedings of the National Academy of Sciences* 111(29): E2957–E2966.

- Hellberg, M. E., R. S. Burton, J. E. Neigel & S. R. Palumbi, 2002. Genetic assessment of connectivity among marine populations. *Bulletin of Marine Science* 70: 273–290.
- Heled, J., 2010. Extended Bayesian skyline plot tutorial. <http://beast-mcmc.googlecode.com/svn-history/r3936/trunk/doc/tutorial/EBSP/ebps-tut.pdf>. Accessed 24 August 2020.
- Heled, J. & A. J. Drummond, 2008. Bayesian inference of population size history from multiple loci. *BMC Evolutionary Biology* 8: 289.
- Herbert, T. D., K. T. Lawrence, A. Tzanova, L. C. Peterson, R. Caballero-Gill & C. S. Kelly, 2016. Late Miocene global cooling and the rise of modern ecosystems. *Nature Geoscience* 9: 843–847.
- Hewitt, G., 2000. The genetic legacy of the Quaternary ice ages. *Nature* 405: 907–913.
- Hirschfeld, M., C. Dudgeon, M. Sheaves & A. Barnett, 2021. Barriers in a sea of elasmobranchs: From fishing for populations to testing hypotheses in population genetics. *Global Ecology and Biogeography* 00: 1–17.
- Instituto Chico Mendes de Conservação da Biodiversidade, 2018. Volume VI - Peixes. In: Instituto Chico Mendes de Conservação da Biodiversidade (org) Livro Vermelho da Fauna Brasileira Ameaçada de Extinção. ICMBio, Brasília
- Iriarte, P. J. F., M. P. Alonso, D. E. Sabadin, P. A. Arauz & C. M. Iudica, 2011. Phylogeography of weakfish *Cynoscion guatucupa* (Perciformes: Sciaenidae) from the southwestern Atlantic. *Scientia Marina* 75: 701–706.
- Karl, S. A., A. L. Castro & R. C. Garla, 2012. Population genetics of the nurse shark (*Ginglymostoma cirratum*) in the western Atlantic. *Marine Biology* 159: 489–498.
- Kearse, M., R. Moir, A. Wilson, S. Stones-Havas, M. Cheung, S. Sturrock & A. Drummond, 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28: 1647–1649.
- Leigh, J. W. & D. Bryant, 2015. POPART: full-feature software for haplotype network construction. *Methods in Ecology and Evolution* 6: 1110–1116.
- Lucifora, L. O., V. B. Garcia, R. C. Menni & B. Worm, 2012. Spatial patterns in the diversity of sharks, rays, and chimaeras (Chondrichthyes) in the Southwest Atlantic. *Biodiversity and Conservation* 21: 407–419.
- Ludt, W. B. & L. A. Rocha, 2015. Shifting seas: the impacts of Pleistocene sea-level fluctuations on the evolution of tropical marine taxa. *Journal of Biogeography* 42: 25–38.
- Lutz, V. A., V. Segura, A. I. Dogliotti, D. A. Gagliardini, A. A. Bianchi & C. F. Balestrini, 2010. Primary production in the Argentine Sea during spring estimated by field and satellite models. *Journal of Plankton Research* 32: 181–195.
- Machado, L. F., J. de Souza Damasceno, A. A. Bertoncini, A. P. C. Farro, M. Hostim-Silva & C. Oliveira, 2017. Population genetic structure and demographic history of the spadefish, *Chaetodipterus faber* (Ephippidae) from Southwestern Atlantic. *Journal of Experimental Marine Biology and Ecology* 487: 45–52.
- Machado, R. C., M. C. da Silva Cortinhas, M. C. Proietti & M. Haimovici, 2020. Genetic connectivity of black drum (*Pogonias courbina*) stocks in the southwestern Atlantic Ocean. *Environmental Biology of Fishes* 103(8): 913–926.
- Márquez, A., A. M. Solé-Cava, A. N. Pereira, A. Vasconcellos & E. P. Lessa, 2017. Deep genetic divergence in the Southern kingcroaker *Menticirrhus americanus* in its southernmost distribution. *Brazilian Journal of Oceanography* 65: 515–519.
- Mendonça, F. F., C. Oliveira, O. B. F. Gadig & F. Foresti, 2011. Phylogeography and genetic population structure of Caribbean sharpnose shark *Rhizoprionodon porosus*. *Reviews in Fish Biology and Fisheries* 21: 799–814.
- Mendonça, F. F., C. Oliveira, O. B. F. Gadig & F. Foresti, 2013. Diversity and genetic population structure of the Brazilian sharpnose shark *Rhizoprionodon lalandii*. *Aquatic Conservation: Marine and Freshwater Ecosystems* 23: 850–857.
- Miranda, L. V. & M. Haimovici, 2007. Changes in the population structure, growth and mortality of striped weakfish *Cynoscion guatucupa* (Sciaenidae, Teleostei) of southern Brazil between 1976 and 2002. *Hydrobiologia* 589: 69–78.
- O'Brien, S. M., V. F. Gallucci & L. Hauser, 2013. Effects of species biology on the historical demography of sharks and their implications for likely consequences of contemporary climate change. *Conservation Genetics* 14: 125–144.
- Oddone, M., C. A. Awruch, R. Barreto, P. Charvet, G. E. Chiraramonte, J. M. Cuevas, et al., 2019. *Squatina guggenheim*. The IUCN Red List of Threatened Species 2019: e.T130393378A130393975.
- Odebrecht, C. & J. P. Castello, 2001. The convergence ecosystem in the Southwest Atlantic. In Seeliger, U. & B. Kjerfve (eds), *Coastal marine ecosystems of Latin America* Springer, Berlin: 147–165.
- Ovenden, J. R., 2013. Crinkles in connectivity: combining genetics and other types of biological data to estimate movement and interbreeding between populations. *Marine and Freshwater Research* 64(3): 201–207.
- Piola, A. R., E. J. Campos, O. O. Möller Jr., M. Charo & C. Martinez, 2000. Subtropical shelf front off eastern South America. *Journal of Geophysical Research: Oceans* 105: 6565–6578.
- R Core Team, 2020. R: A language and Environment for Statistical Computing, R Foundation For Statistical Computing, Vienna
- Rabassa, J., A. M. Coronato & M. Salemme, 2005. Chronology of the Late Cenozoic Patagonian glaciations and their correlation with biostratigraphic units of the Pampean region (Argentina). *Journal of South America Earth Sciences* 20: 81–103.
- Rambaut, A., A. J. Drummond, D. Xie, G. Baele & M. A. Suchard, 2018. Posterior summarization in Bayesian phylogenetics using Tracer 1.7. *Systematic Biology* 67(5): 901.
- Ramírez-Amaro, S., D. Ramírez-Macías, R. Vázquez-Juárez, S. Flores-Ramírez, F. Galván-Magaña & J. N. Gutiérrez-Rivera, 2017. Population structure of the Pacific angelshark (*Squatina californica*) along the northwestern coast of Mexico based on the mitochondrial DNA control region. *Ciencias Marinas* 43: 69–80.

- Ramos-Onsins, S. E. & J. Rozas, 2002. Statistical properties of new neutrality tests against population growth. *Molecular Biology and Evolution* 19: 2092–2100.
- Rodrigues, R., S. Santos, M. Haimovici, U. Saint-Paul, I. Sampaio & H. Schneider, 2014. Mitochondrial DNA reveals population structuring in *Macrondon atricauda* (Perciformes: Sciaenidae): a study covering the whole geographic distribution of the species in the southwestern Atlantic. *Mitochondrial DNA* 25(2): 150–156.
- Rozas, J., A. Ferrer-Mata, J. C. Sánchez-DelBarrio, S. Guirao-Rico, P. Librado, S. E. Ramos-Onsins & A. Sánchez-García, 2017. DnaSP 6: DNA sequence polymorphism analysis of large data sets. *Molecular Biology and Evolution* 34: 3299–3302.
- Sandoval-Castillo, J., A. Rocha-Olivarez & E. F. Balford, 2004. Cryptic isolation of Gulf of California shovelnose guitarfish evidenced by mitochondrial DNA. *Marine Biology* 145: 983–988.
- Santos, S., T. Hrbek, I. P. Farias, H. Schneider & I. Sampaio, 2006. Population genetic structuring of the king weakfish, *Macrondon ancylodon* (Sciaenidae), in Atlantic coastal waters of South America: deep genetic divergence without morphological change. *Molecular Ecology* 15: 4361–4373.
- Santos, T. P., D. O. Lessa, I. M. Venancio, C. M. Chiessi, S. Multiza, H. Kuhnert, et al., 2017. Prolonged warming of the Brazil Current precedes deglaciations. *Earth Planetary Science Letters* 463: 1–12.
- Stadler, T., 2010. Sampling-through-time in birth–death trees. *Journal of Theoretical Biology* 267(3): 396–404.
- Stelbrink, B., T. von Rintelen, G. Cliff & J. Kriwet, 2010. Molecular systematics and global phylogeography of angel sharks (genus *Squatina*). *Molecular Phylogenetics and Evolution* 54(2): 395–404.
- Toomey, L., D. Welsford, S. A. Appleyard, A. Polanowski, C. Faux, B. E. Deagle, ... & S. Jarman, 2016. Genetic structure of Patagonian toothfish populations from otolith DNA. *Antarctic Science* 28(5): 347–360. <https://doi.org/10.1017/S0954102016000183>.
- Vaidya, G., D. J. Lohman & R. Meier, 2011. SequenceMatrix: concatenation software for the fast assembly of multi-gene datasets with character set and codon information. *Cladistics* 27: 171–180.
- Vasconcellos, A. V., D. Lima, F. Bonhomme, M. Vianna & A. M. Solé-Cava, 2015. Genetic population structure of the commercially most important demersal fish in the Southwest Atlantic: the whitemouth croaker (*Micropogonias furnieri*). *Fisheries Research* 167: 333–337.
- Vaz-dos-Santos, A. M., C. L. D. B. Rossi-Wongtschowski & J. L. D. Figueiredo, 2009. *Merluccius hubbsi* (Teleostei: Merlucciidae): stock identification based on reproductive biology in the South-Southeast Brazilian region. *Brazilian Journal of Oceanography* 57: 17–31.
- Viñas, M. D., R. M. Negri, F. C. Ramírez & D. Hernández, 2002. Zooplankton assemblages and hydrography in the spawning area of anchovy (*Engraulis anchoita*) off Rio de la Plata estuary (Argentina–Uruguay). *Marine and Freshwater Research* 53: 1031–1043.
- Vögler, R., A. C. Milessi & R. A. Quiñones, 2008. Influence of environmental variables on the distribution of *Squatina guggenheim* (Chondrichthyes, Squatinidae) in the Argentine-Uruguayan Common Fishing Zone. *Fisheries Research* 91: 212–221.
- Vooren, C. M. & S. Klippel, 2005. Biologia e status de conservação dos cações-anjo *Squatina guggenheim*, *S. occulta* e *S. argentina*. In Vooren CM, Klippel S (ed) *Ações para a conservação de tubarões e raias no sul do Brasil*. Igaré, Porto Alegre: 262.
- Vooren, C. M., S. Montealegre-Quijano, R. P. T. Lessa, R. R. P. Barreto, J. M. R. Soto, J. E. Kotas & R. A. dos Santos, 2018. *Squatina guggenheim* Marini, 1936. In Instituto Chico Mendes de Conservação da Biodiversidade (org) *Livro Vermelho da Fauna Brasileira Ameaçada de Extinção: Volume VI – Peixes*. ICMBio, Brasília: 1112–1116.

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