Evolutionary history of *Bathygobius* (Perciformes: Gobiidae) in the Atlantic biogeographic provinces: a new endemic species and old mitochondrial lineages

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The high diversity and distribution of *Bathygobius* Bleeker, 1878 makes it an excellent group to study evolution patterns within the Atlantic. Thus, the aims of this work were to investigate the taxonomic status, geographical distribution and evolutionary history of *Bathygobius* in the Atlantic and to examine the genetic structure and demographic and phylogeographic history of two species widely distributed in the Atlantic, *Bathygobius soporator* and *Bathygobius geminatus*. Our results indicate that a new insular species, **Bathygobius brasiliensis sp. nov.**, can be found in the Western Atlantic, which diverged from its sister species, *Bathygobius antilliensis*, around 3.03 Mya [cytochrome *c* oxidase I (*COI*) d = 0.063; cytochrome *b* (cyt *b*) d = 0.074]. There are several old mitochondrial lineages and limited gene flow among major biogeographic provinces of both *B. soporator* and *B. geminatus* (*COI*: overall Φ_{ST} = 0.32, P < 0.05; overall Φ_{ST} = 0.31, P < 0.05, respectively). We discuss how the Atlantic biogeographical barriers appear to have influenced the formation of the sister species of *Bathygobius*, and we formulate hypotheses on how the intermittent nature of biogeographical barriers, coupled with the ecological and biological traits of species and ecological and hydrological characteristics of the provinces, shaped the genetic structure and phylogeography of *B. soporator* and *B. geminatus*.

ADDITIONAL KEYWORDS: *Bathygobius brasiliensis* – genetic structure – gobies – Noronha frillfin goby – phylogenetics – phylogeography.

INTRODUCTION

Phylogeographic studies of reef fishes have contributed to understanding the evolution and distribution patterns observed in the Atlantic fauna (e.g. Briggs & Bowen, 2012; Bowen *et al.*, 2013; Cowman, 2014). Biogeographical barriers or filters had a significant role in generating and maintaining biodiversity in the Atlantic Ocean (Floeter *et al.*, 2008). The two hard biogeographical barriers most important for the speciation process of the marine New World fauna were the Terminal Tethyan Event (TTE) and the final closure of the Isthmus of Panama (IOP). The TTE occurred after the uplift of the Red Sea land bridge, separating the tropical faunas of the East Pacific/Atlantic and the West-Central Pacific/Indian Ocean *c*. 12–18 Mya (Dercourt *et al.*, 1986). The IOP, a consequence of the collision between South America and the Panama Block, isolated the faunas of the Caribbean and the Eastern Pacific. This event was considered to have taken place *c*. 3.5 Mya (Coates & Obando, 1996), but this age may be an underestimate: recent studies have suggested an early and complex emergence of the Isthmus, where the initial collision of the Panama Block and

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South America occurred *c*. 23–25 Mya, and the end of the exchange of deep and intermediate waters between the Caribbean and the Pacific occurred *c.* 10 Mya (Farris *et al.*, 2011; Bacon *et al.*, 2015; Montes *et al.*, 2015).

Within the Atlantic Basin, there are three further, albeit soft (weak or intermittent), biogeographical barriers: the mid-Atlantic barrier, the Amazon discharge and the Benguela barrier. It is clear that the presence of those barriers not only restricts dispersal but also allows occasional crossings, leading to the establishment of new populations and species in allopatry or parapatry (Floeter *et al.*, 2008). The mid-Atlantic Barrier began to form following the separation of Africa and South America, around 84 Mya (Pittman *et al.*, 1993 cited in Floeter *et al.*, 2008). The second barrier was the freshwater discharge of the Orinoco and Amazon rivers that separated Brazilian and Caribbean reef habitats, around 7 Mya (Figueiredo *et al.*, 2009; Hoorn *et al.*, 2010). Finally, the third soft barrier was the cool Benguela Current that started around 2 Mya, limiting the interchange between faunas of the Atlantic and the southern Indian Oceans (Marlow *et al.*, 2000; see Floeter *et al.*, 2008 for a review).

Marine provinces are delimited on the basis of biogeographical barriers and have distinct biological features (Briggs & Bowen, 2012). In the Western Atlantic, there are four primary provinces: the Carolina (CAL, in the northern Gulf of Mexico from Cape Rojo, Mexico, to Cape Romano, Florida, and on the Atlantic coast from Cape Canaveral, Florida, to Cape Hatteras, North Carolina), the Caribbean (CA, from Bermuda and Cape Canaveral, Florida, to the Amazon River), the Brazilian (BR, from the mouth of the Amazon River south to Santa Catarina, Brazil, including the oceanic islands) and the Argentinian (from Santa Catarina, Brazil, to the Península Valdés, Argentina) provinces. In the Eastern Atlantic, there are ten main provinces, one of which is relevant to this study: the Tropical Eastern Atlantic (TEA, from Cape Verde to Angola) (Floeter *et al.*, 2008; Briggs & Bowen, 2012, 2013). The relationships among provinces in the Atlantic Ocean are complex and reticulated, leading to different patterns in the genetic structure of populations, especially in relation to the degree of genetic heterogeneity among different provinces (Floeter *et al.*, 2008). In reef fishes and invertebrates, for example, several phylogeographic studies have revealed deep (e.g. Carlin, Robertson & Bowen, 2003; Lessios, Kane & Robertson, 2003; Rocha *et al.*, 2005), some (e.g. Rocha *et al.*, 2002, 2005; Bowen *et al.*, 2006) and even no (e.g. Rocha *et al.*, 2002; Bowen *et al.*, 2006) significant genetic structuring among biogeographic provinces of the Atlantic.

The family Gobiidae, with nearly 2000 described species, is one of the most abundant fish families with species associated with coral reefs (Herler, Munday & Hernaman, 2011). In the Western Atlantic, there are 29 genera of gobies (Floeter *et al.*, 2008; Tornabene *et al.*, 2016b), and, in recent years, several new species have been described for this region (e.g. Tornabene *et al.*, 2010; Victor, 2014; Baldwin & Robertson, 2015; Van Tassell *et al.*, 2015; Tornabene, Robertson & Baldwin, 2016a). In addition to those new species, genetic differences have been observed among Caribbean populations in some goby species (e.g. Rüber *et al.*, 2003; Taylor & Hellberg, 2003, 2006; Victor, 2014), while in others no genetic differentiation was found (e.g. Victor, 2014). Because of the high diversity, distribution and biological features of its species, the pantropical/subtropical genus *Bathygobius* Bleeker, 1878 can be very useful to study patterns of evolution and speciation within and among ocean basins. Species of *Bathygobius* are small and common in sheltered and exposed shallow rocky or sandy shorelines, reef crests, mangroves, seagrass beds, rock jetties and seawalls (Garzón-Ferreira & Acero, 1992; Tornabene *et al.*, 2010). The adults of *Bathygobius* are relatively sedentary, so their dispersal depends on their larvae, which in some species can live for up to 31 days in the water column, and on their interactions with ecological and physical processes (Tavolga, 1954; Peters, 1983).

Currently, 28 species of *Bathygobius* are recognized, six of which occur in the Western Atlantic (Tornabene & Pezold, 2011): *Bathygobius antilliensis* Tornabene, Baldwin & Pezold, 2010; *Bathygobius curacao* (Metzelaar, 1919); *Bathygobius geminatus* Tornabene, Baldwin & Pezold, 2010; *Bathygobius lacertus* (Poey, 1860); *Bathygobius mystacium* Ginsburg, 1947; and *Bathygobius soporator* (Valenciennes, 1837). The low morphological diversity in some gobiid genera hampers delimiting their species through morphology alone (Akihito *et al.*, 2000). This problem is particularly important in *Bathygobius*, whose species often have overlapping meristic counts and morphometric measurements (Tornabene *et al.*, 2010). Several studies have been performed on the systematics and phylogeny of Western Atlantic *Bathygobius*, especially in the Caribbean region (Ginsburg, 1947; Tornabene *et al.*, 2010; Tornabene & Pezold, 2011), but the species from the Brazilian province have not been examined in detail (Lima *et al.*, 2005; Lima-Filho *et al.*, 2016). Given that some Caribbean *Bathygobius* species were detected through genetic analyses, studies comparing those species with individuals from the Southwestern Atlantic are necessary to ascertain their geographical distributions. Morphological, cytogenetic and molecular analyses of specimens of *Bathygobius* from the Equatorial West Atlantic oceanic islands of Atol das Rocas and Fernando de Noronha indicated that the taxonomic status of those gobies should be reviewed (Lima-Filho *et al.*, 2012, 2016; Lima *et al.*, 2005; Tornabene *et al.*, 2010). Moreover, the inspection of a photograph of a *Bathygobius* specimen from Brazil on

FishBase suggested that *B. geminatus* could occur in Brazil (Tornabene *et al.*, 2010), and although a recent molecular analysis confirmed that that species was found off the coast of Rio Grande do Norte State, Brazil (Lima-Filho *et al.*, 2016), the limits of its geographic distribution remain unclear. Mitochondrial haplogroups, or lineages like those defined by Tornabene & Pezold (2011), have been recognized in the North Atlantic within *B. soporator* and *B. geminatus* (Tornabene *et al.*, 2010; Tornabene & Pezold, 2011), but detailed genetic analyses are necessary to determine which lineages occur in the Southwestern Atlantic.

Hence, the aims of this study were (1) to use mitochondrial and nuclear markers to investigate the taxonomic status, geographical distribution and evolutionary history of *Bathygobius* species from the Western Atlantic and (2) to use mitochondrial sequences to assess how the barriers between major biogeographic provinces influence the population structure and demographic history of *B. soporator* and *B. geminatus*, the two species of the genus with the largest distribution in the Atlantic.

MATERIAL AND METHODS

SAMPLE COLLECTION AND DNA PROCESSING

For the molecular analyses, 149 specimens were collected from nine localities in Brazil, two localities in Colombia and one locality in Barbados. Two specimens from the Bahamas and two from Atol das Rocas from the study of Lima *et al.* (2005) were also included (Supporting Information, Table S1 in Appendix S1). Specimens were collected from tidal ponds using hand nets and stored in ethanol. Specimen identifications were based on taxonomic keys and descriptions from Tornabene *et al.* (2010).

Total genomic DNA was obtained from muscle tissue by a modified salting-out protocol (Rodríguez-Rey, Solé-Cava & Lazoski, 2013). Fragments of three genes were amplified using the primers: BOL–F1 (5′-TCAACYAATCAYAAAGATATYGGCAC-3′) and BOL–R1 (5′-ACTTCYGGGTGRCCRAARAATCA-3′) for the mitochondrial cytochrome *c* oxidase subunit I (*COI*) gene (Rocha *et al.*, 2008b); FishcytB–F (5′-ACCACCGTTGTTATTCAACTACAAGAAC-3′) and TruccytB–R (5′-CCGACTTCCGGATTACAAGACCG-3′) for the mitochondrial cytochrome *b* (cyt *b*) gene (Sevilla *et al.*, 2007); and RAG1–F1 (5′-CTGAGCTGCAGTCAGTACCATAAGATGT-3′) (López, Chen & Ortí, 2004) and RAG1–Ra (5′-CGGGCRTAGTTCCCRTTCATCCTCAT-3′) (Tornabene & Pezold, 2011) for the nuclear recombination activating gene 1 (*RAG1*). Amplification reactions included ~30 ng of gDNA, 1 U of GoTaq Flexi DNA polymerase (Promega, Madison, WI, USA), 3 µL of 5×

Green GoTaq Flexi Buffer, 0.2 mM of dNTPs, 2.5 mM of MgCl$_2$ and 0.3 µM of each primer, in a final volume of 15 µL. Polymerase chain reactions (PCR) were carried out using an initial denaturation at 95 °C for 5 min; 35 cycles of denaturation at 94 °C for 30 s, annealing at 50 °C for 35 s and extension at 72 °C for 2 min (FishcytB–F/TruccytB–R), 80 s (RAG1–F1/RAG1–Ra) or 45 s (BOL–F1/BOL–R1), and a final extension at 72 °C for 7 min. PCR products were purified using the Agencourt AMPure PCR purification kit in the epMotion 5075 Automated Pipetting System (Eppendorf, Hamburg, Germany). Sequences of purified PCR products were obtained in both directions using an ABI 3500 automated DNA sequencer (Applied Biosystems, Waltham, MA, USA). Sequences were edited using SEQMAN II 4.0 (DNAstar Inc.) and have been deposited in GenBank (Accession numbers *COI*: KT357888–KT358034; cyt *b*: KT358035–KT358182; *RAG1*: KT357861–KT357887).

PHYLOGENETIC ANALYSES OF *BATHYGOBIUS*

New sequences of the three genes from 27 specimens, together with sequences obtained from the works of Tornabene *et al.* (2010) and Tornabene & Pezold (2011), were used to investigate the status of *Bathygobius* species from the Western Atlantic (Supporting Information, Table S2). *Istigobius decoratus* was chosen as outgroup because of it is closely related to *Bathygobius* (Agorreta *et al.*, 2013; Thacker, 2015).

Alignments of each gene data set were conducted using the CLUSTALW algorithm implemented in MEGA 5.04 (Tamura *et al.*, 2011) and checked manually for misalignments. Gaps were not observed in any of the alignments. Nucleotide divergences were estimated in MEGA, using the Kimura 2-parameter distance model (K2P) (Kimura, 1980).

For the phylogenetic analyses, three different data sets were used: the nuclear (*RAGI*) data set; the mitochondrial (*COI* + cyt *b*) data set and the mitochondrial-nuclear data set, concatenated with the help of MESQUITE 2.74 (Maddison & Maddison, 2010). The best nucleotide substitution model for each gene and data set was determined according to the Bayesian information criterion as implemented in MEGA. The selected model for all genes or data sets was the HKY + I + G.

Phylogenetic trees were obtained using Bayesian inference (BI) and maximum likelihood (ML) methods. The BI phylogeny was performed in MRBAYES 3.2.0 (Ronquist *et al.*, 2012), and the concatenated data sets were partitioned by gene. For each data set, two independent analyses of four Markov chain Monte Carlo (MCMC) were run, each with 15 × 10^6 generations and sampled every 1000th generations. For each

analysis, the first 25% of trees sampled were discarded as burn-in and a 50% majority-rule consensus tree was obtained. The ML phylogenetic analysis was carried out with PHYML 3.0 (Guindon *et al.*, 2010) using the nearest neighbour interchange method for optimizing tree search. Node support was evaluated with 1000 bootstrap replicates.

Two species delimitation methods were implemented on the *COI* and cyt *b* data sets: the automatic barcode gap discovery (ABGD; Puillandre *et al.*, 2012) and the Poisson tree processes (PTP; Zhang *et al.*, 2013). The ABGD is a distance method that uses the gap observed between intraspecific diversity and interspecific diversity (barcode gap) to partition the data, while the PTP is a method that infers the putative species boundaries using non-ultrametric phylogenies (Puillandre *et al.*, 2012; Zhang *et al.*, 2013). For the ABGD test, the number of species on the initial partition was inferred using the K2P distance in the web interface (http://wwwabi.snv.jussieu.fr/public/abgd/abgdweb.html, last accessed 2 April 2017). For the PTP test, the species were inferred on the ML trees in the web interface of Bayesian PTP (bPTP) (http://species.h-its.org/ptp/, last accessed 2 April 2017) using 5×10^5 MCMC generations.

The Bayesian binary MCMC (BBM) method was used to reconstruct ancestral distributions as implemented in RASP 3.2 (Yu *et al.*, 2014, 2015). For the analysis, six geographical areas were used to define the species distributions: Northwestern Atlantic, Southwestern Atlantic, Eastern Atlantic, Eastern Pacific, Indo-West Pacific and Western Indian Ocean. Each Operational Taxonomic Unit was assigned to one or more of the areas based on sampling locality or the known current distribution of the species. The combined output from the two MrBayes runs of the mitochondrial-nuclear data set (27 000 trees retained after a burn-in of 10%) served as input file. The maximum number of ancestral areas per node was set to six; the F81 model was chosen and 10 chains were run with 1×10^6 generations, sampling every 100 generations and a burn-in of 1000 samples.

To estimate divergence times, 37 outgroup taxa representing the Gobioidei diversity and 1 outgroup taxon representing the Apogonoidei were included in the mitochondrial-nuclear data set (Supporting Information, Table S3). For the analysis, four calibration points were used. The first three points, derived from a larger-scale analysis of Gobiiformes done by Thacker (2014), were as follows: the time since the most recent common ancestor (tMRCA) for the family Gobiidae (50 Myr), Gobionellidae (48 Myr) and Gobiidae + Gobionellidae (55 Myr). The fourth point, the tMRCA of *Kribia nana* and *Bostrychus zonatus*, was set to 23 Myr based on a well-preserved fish fossil with otoliths of an upper Oligocene Butidae species, which was hypothesized to be a close relative of

Kribia (Gierl *et al.*, 2013). The priors for the three first points were calibrated using a normal distribution (mean at the calibration age; SD, 1), while the fourth point was calibrated using a log-normal distribution (mean = 11.5; SD, 0.75; offset, 23). Similar calibrations were implemented in previous studies (Thacker, 2015; Tornabene *et al.*, 2016b).

Divergence time inference was conducted in BEAST 1.8.2 (Drummond & Rambaut, 2007), using a relaxed clock uncorrelated log-normal approach with a birth–death process for the tree prior. A substitution model was set for each gene (*COI*: GTR + I + G; cyt *b*: HKY + I + G; RAG: HKY + I), while two clock models were set for the data set (one for both mitochondrial genes combined and another for the nuclear gene) and single tree model. Two independent MCMC simulations of 10^8 generations were run by sampling every 1000th generations, with the first 25% discarded as burn-in. Results were examined with TRACER 1.5 (Rambaut & Drummond, 2007) to assess the effective sample sizes and the convergence among Markov chains. The analyses were combined using LOGCOMBINER 1.8.2, and a maximum clade credibility tree was constructed using TREEANNOTATOR 1.8.2 (Drummond & Rambaut, 2007). The analyses with BEAST and MRBAYES were conducted on the CIPRES Science Gateway (Miller, Pfeiffer & Schwartz, 2010).

PHYLOGEOGRAPHY AND POPULATION STRUCTURE OF *B. SOPORATOR* AND *B. GEMINATUS*

The phylogeographic and population structure analyses of *B. soporator* and *B. geminatus* were based on the *COI* and cyt *b* sequences obtained here, supplemented by Tornabene *et al.* (2010) and Tornabene & Pezold (2011) (Supporting Information, Table S4).

Nucleotide (π) and haplotype (h) diversities and their variances were estimated for each locality using DNASP 5 (Librado & Rozas, 2009). Pairwise genetic divergences between populations were estimated using Φ_{ST} statistics, and population structure was examined through an analysis of molecular variance (AMOVA) using ARLEQUIN 3.5.1.2 (Excoffier & Lischer, 2010), excluding localities with $n = 1$. The statistical significance of estimates was assessed by 10^4 permutations. A false discovery rate (Benjamini & Hochberg, 1995) correction was used to adjust significance levels to account for multiple simultaneous tests, using the Excel spreadsheet created by Pike (2011). The genealogical relationships among haplotypes were assessed through parsimony haplotype networks and phylogenetic analyses. The networks were constructed using a median-joining algorithm as implemented in the software NETWORK 4.6.1.3 (Bandelt, Forster & Röhl, 1999). BI and ML haplotypes phylogenies were conducted as described above. Two independent analyses

of four MCMC each were run with 2×10^6 generations with a sampling frequency of 100 generations for BI analysis. Finally, ancestral distributions were inferred through the BBM method as described previously.

DEMOGRAPHIC ANALYSES OF *B. SOPORATOR* AND *B. GEMINATUS*

The demographic analyses were carried out separately for each of the lineages detected in the analyses using the *COI* sequences, which is the data set with the largest number of samples in all lineages. Tajima's *D* (Tajima, 1989), Fu's F_s (Fu, 1997) and R_2 (Ramos-Onsins & Rozas, 2002) were used to test for deviations from mutation-drift equilibrium in the overall sample. Both Tajima's *D* and Fu's F_s were calculated as implemented in ARLEQUIN. R_2 statistics and their confidence intervals were estimated by coalescence simulations using DNASP. Putative temporal changes in population size of each lineage were investigated through Bayesian skyline plots (BSP) (Drummond *et al.*, 2005) using BEAST. A substitution rate of 1.9% Myr^{-1} for the *COI* gene (Keith *et al.*, 2011) was employed, using a HKY model and two molecular clock approaches: strict and relaxed (uncorrelated log-normal prior). For each analysis, two independent MCMC runs of 30×10^6 generations were performed, sampling every 1000th generations, with the first 10% of each run discarded as burn-in. The analyses for each lineage were combined using LOGCOMBINER 1.7.5 (Drummond & Rambaut, 2007), and the results were summarized in piecewise-constant BSP using TRACER. The Bayes factor (BF) implemented in TRACER was used to compare the molecular clock models employed in the BSPs (Suchard, Weiss & Sinsheimer, 2001).

MORPHOLOGICAL ANALYSES

One hundred and fifty voucher specimens from Fernando de Noronha and Atol das Rocas were analysed and compared with all other species of the Western Atlantic. The list of the voucher specimens can be found in Supporting Information, Appendix S3. All measurements were taken with digital calipers, and counts of procurrent caudal-fin rays were made from digital radiographs of specimens. Descriptions of pigmentation are based on fresh and recently preserved specimens. The dark spots on postorbital, temporal and shoulder regions described for the new species are the same markings shown by Miller & Stefanni (2001: Fig. 4) and cited by Tornabene *et al.* (2010) as occurring in the body as follows: postorbital blotch – posterior to the eye, anterior to a vertical through posterior margin of preopercle; temporal marking – directly above posterodorsal corner of preopercle; and shoulder spot – directly above posterodorsal corner of

operculum. Meristic counts and measurements follow Miller & Smith (1989) and the institutional abbreviations follow Sabaj Pérez (2014).

RESULTS

PHYLOGENETIC ANALYSES OF *BATHYGOBIUS*

Alignments of 573 bp *COI*, 1065 bp cyt *b* and 780 bp *RAG1* sequences were obtained from 51 individuals. The trees recovered from the three data sets had very similar topologies. Hence, we decided to perform the subsequent analyses using the concatenated mitochondrial-nuclear data set, which produced highly resolved trees both with ML or BI analyses (Fig. 1).

Our analyses suggest that at least seven *Bathygobius* species exist in the Western Atlantic: *B. antilliensis*, *B. curacao*, *B. geminatus*, *B. lacertus*, *B. mystacium*, *B. soporator* and a new species, island restricted (Fernando de Noronha and Atol das Rocas), *Bathygobius brasiliensis* sp. nov. (Fig. 1, Supporting Information, Fig. S1 in Appendix S2). The insular *B. brasiliensis* sp. nov. diverged from the most closely related species, *B. antilliensis*, by 6.3% for *COI* and 7.4% for cyt *b* (K2P distances). These levels of genetic divergence are at the lower end of the rank of distance values found between other species of *Bathygobius* (7.0–24.1% for *COI* and 9.0–27.8% for cyt *b*; Table 1), but the new species was objectively identified by the methods implemented for species delimitation. Considering a prior maximum divergence of intraspecific diversity (*P*) of 0.001–0.0215 for the *COI* and of 0.001–0.0359 for the cyt *b*, the ABGD method indicates that the individuals from Fernando de Noronha and Atol das Rocas belong to one of the 13 species identified in the initial partition (Supporting Information, Fig. S2). Similarly, the bPTP method identified that these individuals belong to a distinct species, with a posterior probability of 0.25 for the *COI* and of 0.82 for cyt *b*. Although a low support for the COI was observed, in all trees sampled via MCMC the individuals of *B. brasiliensis* sp. nov. and *B. antilliensis* formed reciprocally monophyletic groups. The posterior probability observed when the two groups were considered conspecific was close to zero (Supporting Information, Figs S3 and S4). *Bathygobius brasiliensis* sp. nov. and *B. antilliensis*, whose ancestral species was distributed in the Northwestern Atlantic (Fig. 1, Table 2), diverged around 3.03 Mya [95% Highest Posterior Density (HPD) 1.55–4.62 Mya; Fig. 2, Table 2].

Two clades of *Bathygobius* were recognized in the phylogenetic analyses (Fig. 1): the '*B. antilliensis* group' – which includes *B. antilliensis* and *B. brasiliensis* sp. nov. from the Atlantic and *Bathygobius ramosus* and *Bathygobius lineatus* from the Pacific – and the '*B. soporator* group' – which comprises the
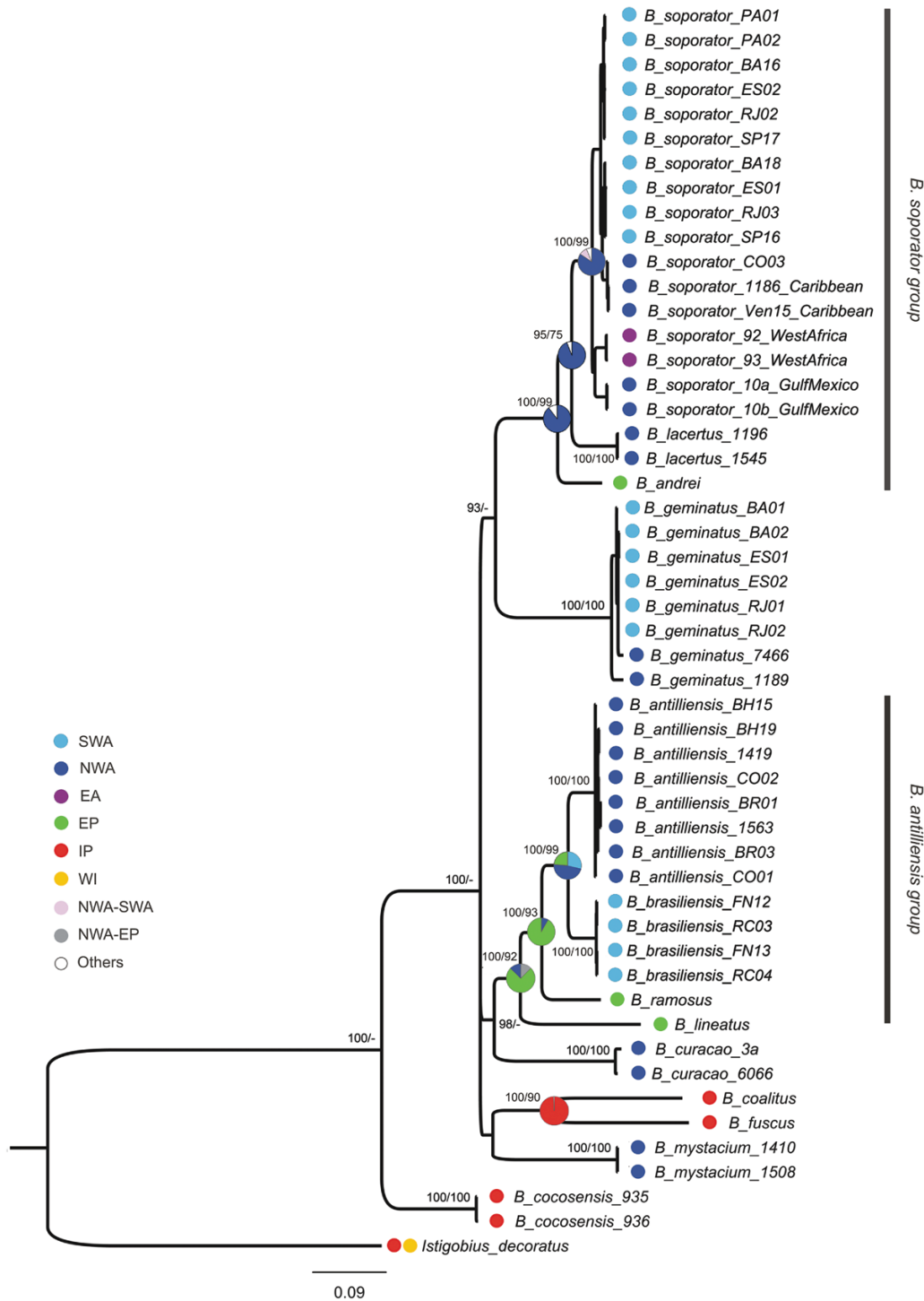
Figure 1. Bayesian phylogeny of *Bathygobius* based on a concatenated mitochondrial-nuclear data set (50% majority-rule consensus tree). Bayesian posterior probability values and ML bootstraps are indicated only for the nodes with over 70% support. *Bathygobius* groups are indicated by vertical bars. Colours denote the distribution as indicated by the embedded key. Current distributions are indicated before the species names. Pie charts of the nodes, with over 75% support for both inference methods, indicate the relative probabilities from ancestral distribution: Southwestern Atlantic (SWA), Northwestern Atlantic (NWA), Eastern Atlantic (EA), Eastern Pacific (EP), Indo-West Pacific (IP) and Western Indian Ocean (WI). For details of the ancestral distribution, see Table 2.

Table 1. Average K2P distance summary for *Bathygobius* species

	Bathygobius andrei	*Bathygobius antilliensis*	*Bathygobius brasiliensis*	*Bathygobius coalitus*	*Bathygobius cocosensis*	*Bathygobius curacao*	*Bathygobius fuscus*	*Bathygobius geminatus*	*Bathygobius lacertus*	*Bathygobius lineatus*	*Bathygobius mystacium*	*Bathygobius ramosus*	*Bathygobius soporator*
Bathygobius andrei		0.19	0.19	0.22	0.22	0.19	0.25	0.23	0.13	0.21	0.20	0.19	0.13
Bathygobius antilliensis	0.20		0.07	0.20	0.21	0.20	0.21	0.23	0.22	0.18	0.20	0.13	0.20
Bathygobius brasiliensis	0.20	0.06		0.21	0.23	0.19	0.22	0.22	0.21	0.18	0.21	0.14	0.20
Bathygobius coalitus	0.18	0.22	0.18		0.24	0.25	0.23	0.22	0.24	0.25	0.22	0.24	0.23
Bathygobius cocosensis	0.20	0.24	0.21	0.20		0.23	0.28	0.27	0.22	0.24	0.20	0.22	0.22
Bathygobius curacao	0.15	0.16	0.16	0.17	0.20		0.24	0.23	0.21	0.22	0.22	0.19	0.19
Bathygobius fuscus	0.18	0.21	0.20	0.19	0.22	0.18		0.23	0.25	0.24	0.24	0.23	0.24
Bathygobius geminatus	0.18	0.15	0.16	0.20	0.22	0.18	0.18		0.23	0.23	0.20	0.23	0.21
Bathygobius lacertus	0.10	0.20	0.18	0.17	0.23	0.16	0.19	0.18		0.22	0.23	0.22	0.09
Bathygobius lineatus	0.20	0.14	0.14	0.20	0.21	0.18	0.21	0.18	0.21		0.23	0.21	0.21
Bathygobius mystacium	0.19	0.19.	0.18	0.20	0.22	0.20	0.19	0.19	0.17	0.14		0.22	0.21
Bathygobius ramosus	0.16	0.11	0.09	0.17	0.22	0.15	0.18	0.17	0.16	0.14	0.19		0.16
Bathygobius soporator	0.07	0.19	0.19	0.20	0.21	0.16	0.19	0.19	0.08	0.21	0.18	0.16	

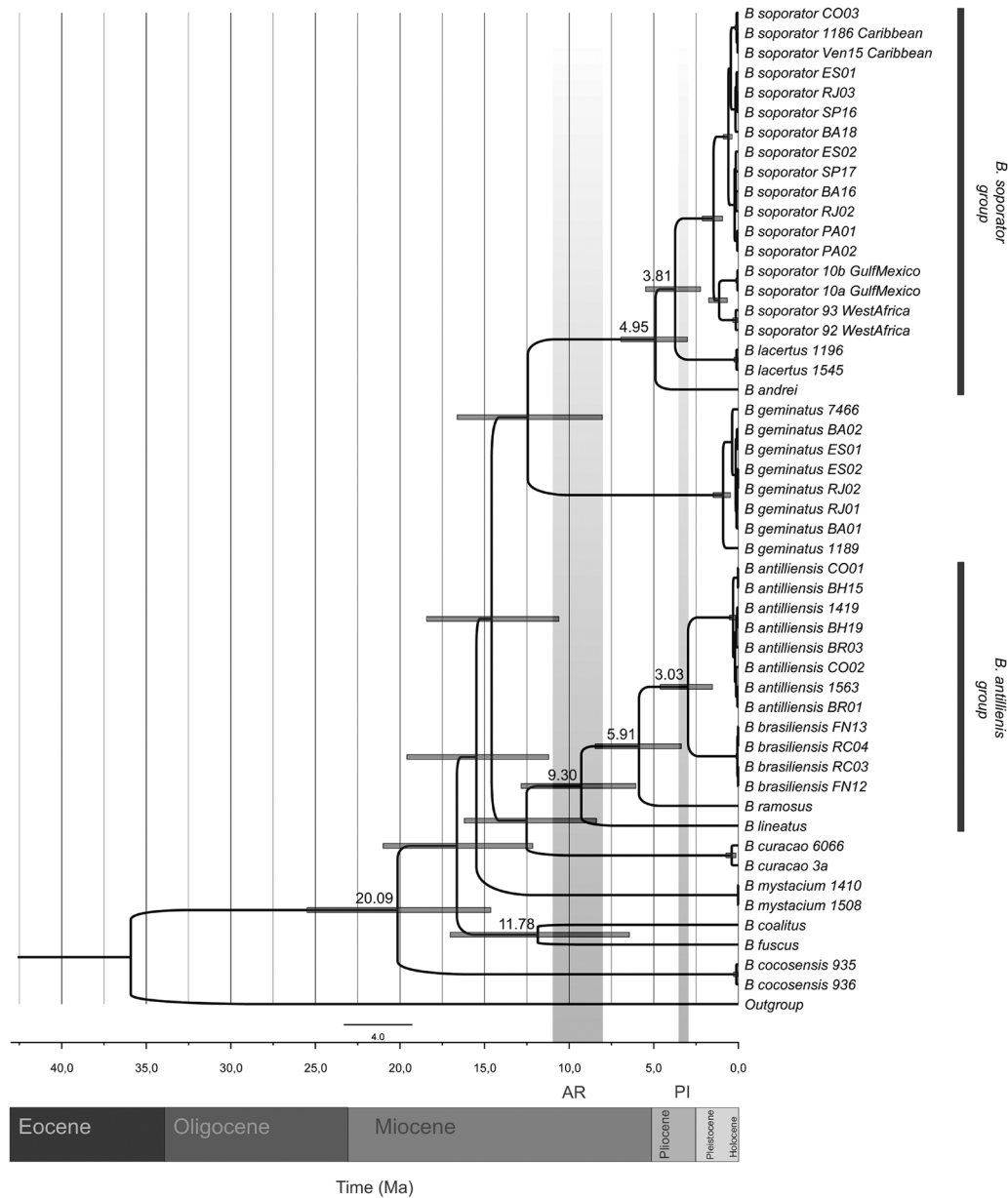Below the diagonal, *COI*; above the diagonal, cyt *b*.

Figure 2. Bayesian estimates of divergence times for *Bathygobius* based on a concatenated mitochondrial-nuclear data set (maximum clade credibility tree). Divergence time in Mya on the nodes with 100% support. The horizontal grey bars indicate 95% credibility intervals of node age estimation. The vertical grey area highlights the establishment of the Amazon River discharge (AR) and the final closure of Panama Isthmus (PI). The outgroups were removed *a posteriori* for clarity.

Atlantic *B. soporator* and *B. lacertus* and *Bathygobius andrei* from the Pacific. The placing of *B. curacao* in the 'B. antilliensis group' as suggested by Tornabene & Pezold (2011) was supported only in the BI analysis of the mitochondrial-nuclear data set. The other analyses failed to resolve the placement of *B. curacao*. Furthermore, *B. curacao* is morphologically very different from the other species of the 'B. antilliensis group' (Tornabene & Pezold, 2011), and thus, we refrain from including this species in that group. The tMRCA of

the thus defined 'B. antilliensis group', based on the mitochondrial-nuclear data set, was of around 9.30 Mya (95% HPD 5.96–12.71 Mya). For the 'B. soporator group', the tMRCA was around 4.95 Mya (95% HPD 3.08–6.88 Mya), and the tMRCA of the whole genus was around 20.09 Mya (95% HPD 14.62–25.39 Mya; Fig. 2, Table 2). The ancestral distributions for the 'B. antilliensis group' and the 'B. soporator group' were the Eastern Pacific and the Northwestern Atlantic, respectively (Fig. 1, Table 2).

Table 2. Age estimates and ancestral distribution reconstructions of *Bathygobius* spp.

Node	Age estimated (95% HPD)	Probabilities for nodal reconstructions
Bathygobius soporator	1.50 (0.96–2.11)	NWA: 0.85; NWA/SWA: 0.09; others: 0.06
Bathygobius soporator / *Bathygobius lacertus*	3.81 (2.30–5.44)	NWA: 0.94; others: 0.06
Bathygobius soporator group	4.95 (3.08–6.88)	NWA: 0.90; others: 0.10
Bathygobius antilliensis / **Bathygobius brasiliensis** sp. nov.	3.03 (1.55–4.62)	NWA: 0.42; SWA: 0.26; EP: 0.21; others: 0.11
Bathygobius antilliensis / **Bathygobius brasiliensis** sp. nov./ *Bathygobius ramosus*	5.91 (3.42–8.45)	EP: 0.83; NWA: 0.08; others: 0.09
Bathygobius antilliensis group	9.30 (5.96–12.71)	EP: 0.74; NWA/EP: 0.13; NWA: 0.12; others: 0.01
Bathygobius coalitus / *Bathygobius fuscus*	11.78 (6.31–17.03)	IP: 0.99; others: 0.01

EP, Eastern Pacific; IP, Indo-West Pacific; NWA, Northwestern Atlantic; SWA, Southwestern Atlantic.

SYSTEMATICS

BATHYGOBIUS BRASILIENSIS CARVALHO-FILHO & DE ARAÚJO **SP. NOV.**

(FIGS 3, S5–S7, TABLES 3, S5)

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Noronha frillfin goby; Bimba de nego

Bathygobius soporator not of Valenciennes 1837 (Boulenger, 1980: 483; Lima *et al*., 2005: 211; Moura, 2003: 251–252; Pinheiro, 2006: 83; Sampaio & Nottingham, 2008: 129; Sazima *et al*., 2013: 63; Soto, 2001: 163; Tornabene *et al*., 2010: 159; Valentim, 2008; Véras & Tolotti, 2011: 107).

Bathygobius aff. *soporator* (Lima-Filho *et al*., 2012: 63; Rangel & Mendes, 2009).

Bathygobius sp. 2 (Lima-Filho *et al*., 2016: not paginated).

Holotype: ZUEC 9637, 70.0-mm standard length (SL), female, Praia do Boldró, Fernando de Noronha (03°51′S; 32°26′W), Brazil, A. Carvalho-Filho, 23 October 2003.

Paratypes: Eight voucher specimens from Fernando de Noronha and one from Atol das Rocas (see Supporting Information, Appendix S3 for details).

Additional non-type specimens: Ninety voucher specimens from Fernando de Noronha and thirty-seven from Atol das Rocas (see Supporting Information, Appendix S3 for details).

Diagnosis: Pectoral-fin rays 18–21 (18: 10; 19: 59; 20: 63; 21: 8); lateral scale rows 34–39 (34: 6; 35: 6; 36: 19; 37: 54; 38: 49; 39: 5); upper jaw length 7.4–10.7% SL (mean 8.9), rarely more than 10% SL (4 out of 54); predorsal squamation reaching anteriorly to or beyond anterior border of postorbital blotch; midline predorsal scales 26–30 (26: 6; 27: 30; 28: 49; 29: 38; 30: 4; not discernible in 13 specimens); pelvic fin falling far of the anus when depressed; cheek and operculum often with a few series of minute scales; first upper pectoral-fin ray usually branch once, occasionally twice; second to fourth branch more than once. Adult males with somewhat rectangular first dorsal fin outline, females and young individuals with a somewhat triangular fin. Small pearly dots were present all over body, head and fins; arranged in horizontal series on body, vertical and horizontal series on fins, and splashed on head specially operculum and preoperculum; yellowish suffusion present everywhere including the outer border of dorsal fins.

Description: For body proportions and meristics see Supporting Information, Table S5. *Bathygobius brasiliensis* sp. nov. agrees in general aspects with the genus as described by Miller & Smith (1989). First dorsal fin VI, second dorsal fin I,9; anal fin I,8; total caudal-fin rays, including procurrent rays, 17 in upper lobe, 15 in lower lobe (one specimen out of 15 with 17/16). Head longer than pectoral-fin length (only 3 of 140 examined specimens had the head shorter). Interorbital width tends to increase relative to eye diameter with increasing SL, anterior part usually flat. Eye diameter greater or equal snout on specimens 24-mm SL or less; snout length longer than eye diameter on specimens over 30-mm SL; snout profile convex to straight, rarely concave. Lower jaw extends to vertical through centre of pupil and often beyond this point. Teeth caniniform, almost straight, in several rows medially; upper jaw with outer row enlarged, teeth spaced, inner rows with smaller packed teeth, a few enlarged among them; lower jaw with outer and inner rows of teeth enlarged, two to three rows of smaller teeth between, usually extending posteriorly as single or double row to

coronoid process, inner row with two pairs of enlarged lateral canines.

First dorsal fin outline somewhat triangular in females and young (last fin-element almost entirely connected to dorsum by fin membrane) to somewhat rectangular in adult males (last fin-element connected only basally to dorsum), fin membrane emarginated between spines, which are eventually free at the tips; posterior rays, when depressed, usually not extending to second dorsal fin origin (extending to in about one third of examined specimens, usually large fishes). Second dorsal fin rear tip extends back from four fifths of distance to upper origin of caudal fin, to above caudal-fin base. Anal fin rear tip extends from one third to two thirds of distance to lower origin of caudal fin. Pectoral fin slightly smaller than head, usually reaching vertical through anterior margin of second dorsal fin and even beyond this point. Pectoral fin with uppermost four or five rays more or less free from membrane, one to four for most to about half extent, five usually only upper branch; all rays branch at least once, first one occasionally twice; second to fourth presenting three or four branches. Pelvic fin oval-shaped to slightly elliptical, rear edge concave. Caudal fin rounded. Body covered with ctenoid scales; those on belly and breast cycloid. Predorsal area and nape with cycloid scales. Cheek with one to three series of minute cycloid scales in lower posterior border, occasionally none; operculum usually with one to seven series of minute cycloid scales, rarely none, in an almost triangular patch in upper anterior corner. Breast covered with often embedded cycloid scales, sometimes only on rear four fifths, about 8–14 along ventral midline.

Coloration: Background colour of body pale, head tan to greenish-brown; small pearly dots arranged in horizontal series on body, vertical and horizontal series on fins and splashed on head specially operculum and preoperculum. These pearly dots are conspicuous on regular to darker specimens except on breeding males, and little discernible in light phased fishes. Ventral portion of trunk with two rows of markings, upper row consisting of seven to eight dark blotches along lateral midline from beneath pectoral fin just before markings on caudal-fin base; lower row usually consisting of three conspicuous dark spots, often a diffuse fourth spot, beginning beneath ventral portion of pectoral fin and terminating just before anal fin; markings on trunk typically more distinct and dorsally connected in small specimens, of lighter colour on body and head, and with small melanophores; dorsal portion of body with four very pale dorsal saddles, usually with a much smaller fifth saddle on posterior caudal peduncle, all barely distinguishable near and below lateral midline; body scales with pearly centre, often suffused with light yellow, giving appearance of narrow horizontal white lines along body, this pattern often less apparent in specimens collected from light substrates; basicaudal markings typically a pair of vertically oriented spots, figuring a three-spot triangle with the last blotch of the trunk upper row of markings; paired spots separate from small spot at origin of dorsal procurrent caudal rays. Fins usually with pearly dots on spines and rays. First dorsal fin translucent either with one to four dark blotches on each element forming one to four distinct longitudinal stripes across lower part of fin, or with one broad, dark brown, longitudinal stripe across fin; second dorsal fin pigmentation similar to first, but stripes usually more distinct than those on first dorsal fin; upper border of dorsal fins often with a yellowish suffusion, much more conspicuous on breeding males. Caudal-fin distal borders usually translucent; branched caudal-fin rays with dark and pearly blotches of varying length, giving appearance of complete or partially broken vertical bars; anal fin lightly pigmented medially, becoming more densely pigmented distally, often with very narrow pale margin at the distal edge; proximal portion of anal fin and overall background colour of dorsal, caudal and pectoral fins, translucent to light brown often tinged with light yellow; fin colour may be darker depending on the substrate over which specimens were collected; pelvic fin dusky to dark, occasionally with a pale rim; pectoral-fin base densely pigmented, often with two concentrations of melanophores forming two distinct blotches, one above the other, continuing onto pectoral-fin rays, fading distally; operculum and preoperculum densely speckled with pearly dots; cheek often with two or three distinct dark blotches, each blotch slightly smaller in diameter than the pupil, anterior cheek blotch typically occurring at posterior margin of lower jaw, second slightly more posterior; third spot of same size sometimes present just posterodorsal to second spot; small, dark, distinct spot present at posteroventral corner of orbit; jaws and snout with scattered white dots, often giving those areas mottled appearance; postorbital blotch either round or oval in shape, its diameter about two third that of pupil; shoulder spot usually oval and larger than postorbital blotch, often iridescent shade of blue in breeding males; abdomen and gular, prepelvic, and branchiostegal regions yellowish to tan densely peppered with melanophores. In life, these fishes show the ability to camouflage with the immediate near surroundings, becoming very light over sand and dark over rocks, but the change is not fast.

Preserved specimens are tan to brownish overall and lose most light dots and markings; on the head, all the pearly dots vanish and melanophores are much more evident on lips, operculum and preoperculum; the yellow shades on fins, head and body also disappear.

Sexual dimorphism: This is the only New World *Bathygobius* species with sexual dimorphism. The outline of the first dorsal fin in large adult males is somewhat rectangular, sometimes really a rectangle,

a feature not shared by females and immature fish, which have the fin outline somewhat triangular (Fig. 3C, D, Supporting Information, Fig. S6). The change from triangular to rectangular outline of the first dorsal fin seems to starts when males are about 36-mm SL.

Comparisons: See Table 3 and Supporting Information, Appendix S3. *Bathygobius brasiliensis* sp. nov. can be distinguished from all other Atlantic and Eastern Pacific species of the genus by the number of predorsal scales (26–30, the highest among all known species of these areas). The presence of scales on operculum and
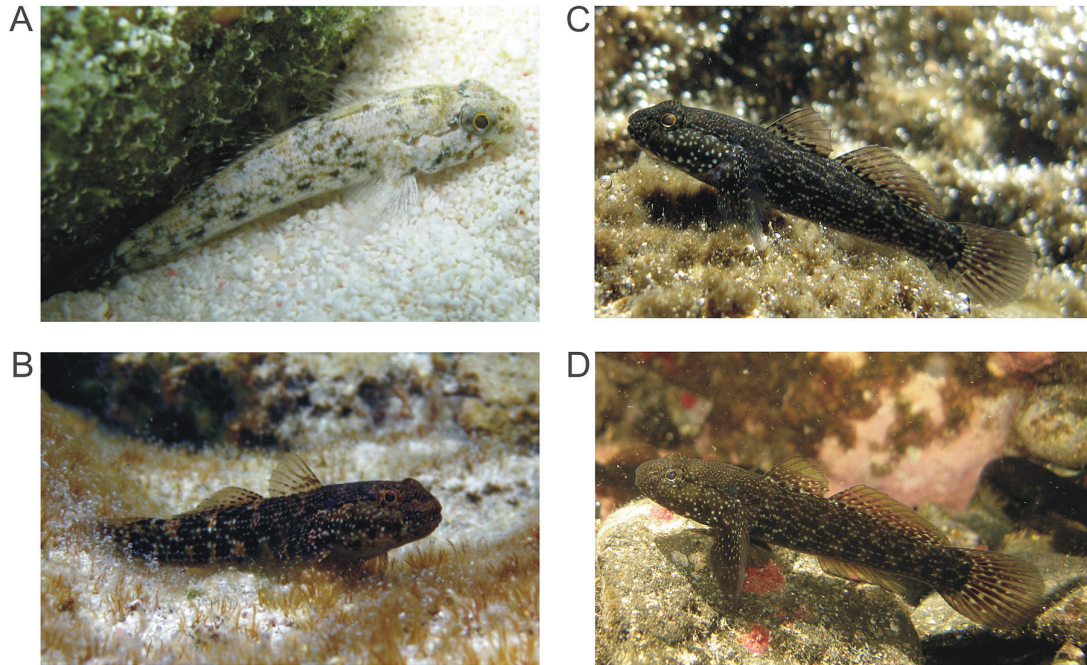

Figure 3. *Bathygobius brasiliensis* **sp. nov.** living on (A) light and (B) dark substrates in Atol das Rocas; adult (C) female and (D) male from Fernando de Noronha. Photos from Atol das Rocas by D. Veras and from Fernando de Noronha by J.-C. Joyeux. Images editing by G.T.R.-R.

Table 3. *Bathygobius* spp. selected characters

Species	Lateral line scales	Predorsal scales	Scales on operculum
Western Atlantic			
***Bathygobius brasiliensis* sp. nov.**	34–39	26–30	Often present
Bathygobius antilliensis	38–42	18–21	Absent
Bathygobius curacao	31–36	17–20	Absent
Bathygobius geminatus	36–38	17–20	Absent
Bathygobius lacertus	38–42	17–20	Absent
Bathygobius mystacium	33–36	17–20	Absent
*Bathygobius soporator**	38–42	19–22	Absent
Eastern Atlantic			
Bathygobius burtoni	33–38	13–18	Absent
Bathygobius casamancus	34–39	14–21	Absent
*Bathygobius soporator**	33–40	16–27	Absent
Eastern Pacific			
Bathygobius andrei	33–36	19–25	Present
Bathygobius lineatus	30–37	18–21	Present
Bathygobius ramosus	32–38	16–20	Absent

*Meristics of *Bathygobius soporator* from Miller & Smith (1989; Eastern Atlantic) and Tornabene *et al.* (2010; Western Atlantic).

preoperculum on 2/3 of the examined specimens also differs it from all other species of the Western Atlantic, which have no scales on those areas. The new species is morphologically quite similar to *B. antilliensis*, with which it was confused by Tornabene *et al.* (2010), but also differs in the smaller length of the upper jaw, 7.4–10.7% SL, rarely more than 10% SL (vs. 9.5–13% SL, rarely less than 11% SL), by the pelvic fin falling far of the anus when depressed (vs. extending to or just falling short of the anus), and by the pectoral fin slightly smaller than the head, usually reaching vertical through anterior margin of second dorsal fin and even beyond that point (vs. pectoral fin slightly longer than head, usually reaching vertical through posterior margin of first dorsal fin, and sometimes beyond this point). Examining recently collected specimens of *B. antilliensis* from Punta Cana, Dominican Republic and Barbados, we observed that this species

also presents the pearly dots on the body, head and fins, even if not so conspicuous, as well as the yellowish outer borders on dorsal fins, being almost identical in general coloration to *B. brasiliensis* sp. nov. Concerning the pectoral-fin upper rays branching, see Discussion under the description of *B. antilliensis* in Tornabene *et al.* (2010).

Habitat: The new species has been collected from reef crests and bottoms, and from shallow tidepools everywhere in Fernando de Noronha and Atol das Rocas, to 3 m deep. It was observed alone, in pairs or in large groups of up to 20 individuals, these often in reef crests with rough waters, and eventually in sheltered sandy bottoms. It co-occurs with several species of small fishes as the Blenniidae *Scartella itajobi*, *Ophioblennius trinitatis* and *Entomacrodus vomerinus*; the Labrisomidae *Malacoctenus* sp., *Labrisomus*
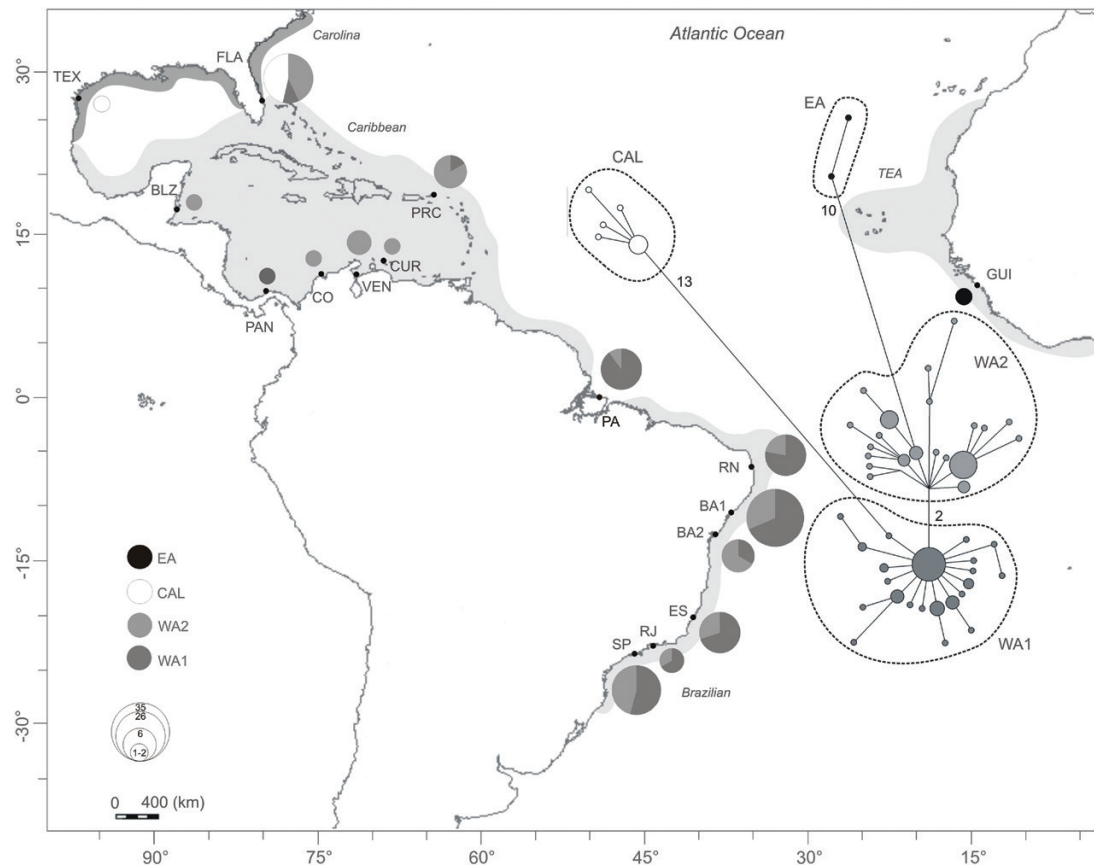

Figure 4. Genealogical relationships among haplotypes of *Bathygobius soporator* and their geographic distribution based on *COI*. In the parsimony median-joining network, sizes of the circles are proportional to the frequency of each haplotype. Line lengths indicate the number of mutations between haplotypes (shortest lines = 1 mutation). Pie charts in the map represent the proportion of each lineage at each location (correspondence between circle sizes and number of individuals is indicated in the legend). Colour coding of network and pie charts corresponds to lineages observed: dark grey, WA1 lineage; light grey, WA2 lineage; white, CAL lineage; black, EA lineage. In the map, the dark grey area corresponds to the warm-temperate biogeographic province (Carolina) and the light grey areas to tropical biogeographic provinces [Caribbean, Brazilian and Tropical East Atlantic (TEA); Briggs & Bowen, 2013].

kalisherae and *Labrisomus conditus*; and the Gobiidae *Coryphopterus glaucofraenum*.

Biological aspects: The new species feeds mainly on small invertebrates, but it may also prey on small fishes. The eggs are guarded by territorial males, and their agonistic behaviour shown in the Supporting Information, Fig. S7.

Distribution: *Bathygobius brasiliensis* is presently known only from the oceanic islands of Fernando de Noronha and Atol das Rocas.

Etymology: The epithet *brasiliensis* refers to the country where the species is found. The English name refers to the Archipelago and the Portuguese name to the name given to this fish by local artisanal fishermen.

PHYLOGEOGRAPHY AND POPULATION STRUCTURE OF *B. SOPORATOR*

The alignment of all *COI* gene sequences (561 bp; $n = 143$) revealed 50 haplotypes (58 polymorphic sites).

COI nucleotide diversity was low to moderate (overall $\pi = 0.010$; range: 0.002–0.016) and haplotype diversity was high (overall $h = 0.92$; range: 0.75–1.00; Table 4). For the cyt *b* gene (1082 bp; $n = 107$), 137 polymorphic sites, distributed among 70 haplotypes, were identified. Levels of cyt *b* gene variation were similar to those observed for *COI* (overall $\pi = 0.011$; range: 0.004–0.015; overall $h = 0.98$; range: 0.87–1.00; Table 4).

Four lineages were observed in the haplotype networks (Fig. 4; Supporting Information, Fig. S8A): one from the Eastern Atlantic limited to the Tropical Eastern Atlantic Province (EA); another from the Northwestern Atlantic restricted to the Carolina Province (CAL); and two sympatric lineages distributed along the Western Atlantic (WA1 and WA2). The grouping of haplotypes in these four lineages is most evident with the cyt *b* gene, where the average pairwise K2P distances between lineages ranged from 1.6% (WA1–WA2) to 4.4% (WA2–EA). For *COI*, distances ranged from 0.8% (WA1–WA2) to 3.1% (WA2–CAL) (Supporting Information, Table S6). The ancestral reconstruction indicated that the

Table 4. Genetic variability in *Bathygobius soporator*

Area	Sampling site	COI				Cyt b			
		n	n_H	h (SD)	π (SD)	n	n_H	h (SD)	π (SD)
East Atlantic	Guinea (GUI)	2	2	1.00 (0.50)	0.004 (0.002)	2	2	1.00 (0.50)	0.005 (0.002)
Northwest Atlantic	Florida (FLA)	28	14	0.86 (0.05)	0.016 (0.001)	–	–	–	–
	Texas (TEX)	2	2	1.00 (0.50)	0.002 (0.001)	2	1	–	–
	Belize (BLZ)	2	1	–	–	–	–	–	–
	Puerto Rico (PRC)	6	5	0.93 (0.12)	0.005 (0.001)	1	1	–	–
	Panama (PAN)	1	1	–	–	–	–	–	–
	Colombia (CO)	1	1	–	–	1	1	–	–
	Venezuela (VEN)	3	3	1.00 (0.27)	0.006 (0.002)	1	1	–	–
	Curaçao (CUR)	1	1	–	–	–	–	–	–
Southwest Atlantic	Pará (PA)	10	6	0.78 (0.14)	0.003 (0.001)	10	7	0.87 (0.11)	0.004 (0.002)
	Rio Grande do Norte (RN)	9	8	0.97 (0.06)	0.005 (0.001)	10	10	1.00 (0.05)	0.009 (0.002)
	Pernambuco (PE)	–	–	–	–	2	2	1.00 (0.50)	0.015 (0.007)
	Bahia 1 (BA1)	35	14	0.85 (0.05)	0.004 (0.001)	35	22	0.96 (0.02)	0.008 (0.001)
	Bahia 2 (BA2)	6	6	1.00 (0.10)	0.007 (0.001)	6	5	0.93 (0.12)	0.012 (0.002)
	Espírito Santo (ES)	10	7	0.91 (0.08)	0.005 (0.001)	10	9	0.98 (0.05)	0.009 (0.002)
	Rio de Janeiro (RJ)	3	3	1.00 (0.27)	0.005 (0.001)	3	3	1.00 (0.27)	0.011 (0.004)
	São Paulo (SP)	24	7	0.75 (0.06)	0.004 (0.001)	24	22	0.99 (0.01)	0.010 (0.001)
Eastern Atlantic lineage (EA)		2	2	1.00 (0.50)	0.004 (0.002)	2	2	1.00 (0.50)	0.005 (0.002)
Carolina lineage (CAL)		14	5	0.51 (0.03)	0.001 (0.001)	2	1	–	–
Western Atlantic 1 lineage (WA1)		69	22	0.79 (0.05)	0.003 (0.0003)	65	42	0.96 (0.0002)	0.003 (0.0002)
Western Atlantic 2 lineage (WA2)		58	21	0.84 (0.04)	0.005 (0.001)	38	25	0.93 (0.03)	0.008 (0.001)
	All samples	143	50	0.92 (0.01)	0.010 (0.001)	107	70	0.98 (0.01)	0.011 (0.001)

π, nucleotide diversity; h, haplotype diversity; n, number of specimens; n_H, number of observed haplotypes.
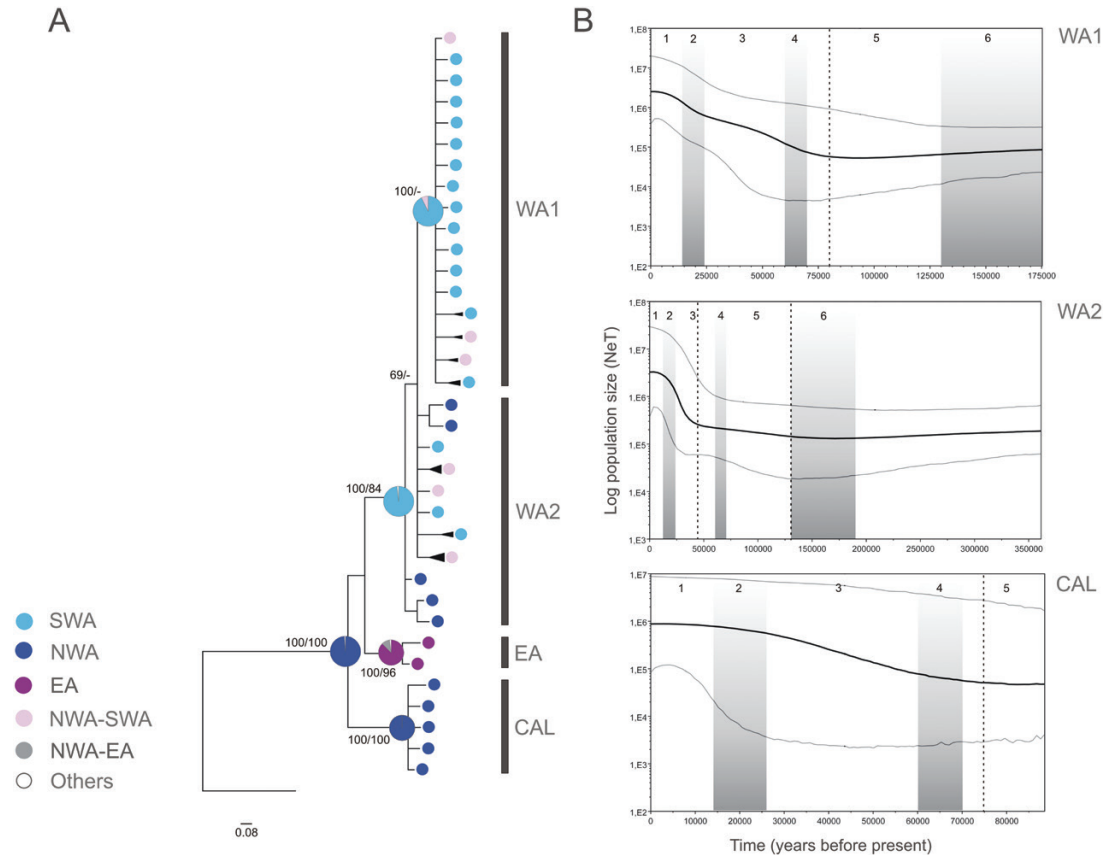
Figure 5. (A) Bayesian phylogenetic tree of haplotypes and (B) BSPs for each lineage of *Bathygobius soporator* based on *COI*. Bayesian posterior probability values and ML bootstraps are indicated only for the nodes with over 60% support (50% majority-rule consensus tree). In the tree, *B. soporator* lineages are indicated by vertical bars. Colours denote the distribution as indicated by the embedded key. Current distributions of each haplotype are indicated at terminals. Pie charts of the nodes, with over 75% support for both inference methods, indicate the relative probabilities from ancestral distribution: Southwestern Atlantic (SWA), Northwestern Atlantic (NWA), Eastern Atlantic (EA) and Eastern Pacific (EP). For details of the ancestral distributions, see Table 6. In the BSP, the median estimate (black solid line) and 95% HPD limits (grey solid line) are indicated. The maximum time is the upper 95% HPD of the root height. Dotted lines correspond to the approximate onset of the expansion events. The main glaciations over the last 200 thousand years are shaded in grey: MIS6 (190–130 ka), MIS4 (70–60 ka) and MIS2 (24–14 ka; Cohen & Gibbard, 2011).

most recent common ancestor of *B. soporator* mtDNA lineages was located in the Northwestern Atlantic (Caribbean + Carolina provinces) (Figs 5A; Supporting Information, Fig. S9A; Table 6).

Population genetics analyses suggested a high genetic differentiation within the Atlantic populations of *B. soporator* (overall Φ_{ST} *COI*: 0.32, $P < 0.05$; cyt *b*: 0.25, $P < 0.05$), indicating restricted gene flow among localities. Significant Φ_{ST} were observed between several pairwise population comparisons (Supporting Information, Table S7). The most likely structuring scenario in the analyses of molecular variance included a hierarchical division in three groups: (1) Eastern Atlantic (GUI), which includes exclusively haplotypes of the EA lineage; (2) Northwestern Atlantic (FLA and TEX) with haplotypes mainly from the CAL and WA2 lineages; and (3) the remaining locations from

the Caribbean to the Southwestern Atlantic (BLZ, PRC, VEN, PA, RN, BA1, BA2, ES, RJ and SP) with haplotypes from the WA1 and WA2 lineages (AMOVA *COI*: Φ_{CT} = 0.46, $P < 0.05$; cyt *b*: Φ_{CT} = 0.78, $P < 0.05$; Supporting Information, Table S8). Additionally, restricted gene flow among localities from the Caribbean and Brazil could also exist since the second most likely structuring scenario separated the two areas (AMOVA *COI*: Φ_{CT} = 0.43, $P < 0.05$; Supporting Information, Table S8).

PHYLOGEOGRAPHY AND POPULATION STRUCTURE OF *B. GEMINATUS*

The alignment of all *COI* gene sequences (614 bp; n = 52) revealed 29 haplotypes (32 polymorphic sites). *COI* nucleotide diversity was low to moderate (overall

Table 5. Genetic variability in *Bathygobius geminatus*

Area	Sampling site	COI				Cyt b			
		n	n_H	h (SD)	π (SD)	n	n_H	h (SD)	π (SD)
Northwest Atlantic	Florida (FLA)	12	6	0.82 (0.009)	0.012 (0.001)	1	1	–	–
	Puerto Rico (PRC)	1	1	–	–	1	1	–	–
Southwest Atlantic	Rio Grande do Norte (RN)	5	4	0.90 (0.161)	0.004 (0.001)	5	5	1.00 (0.126)	0.007 (0.002)
	Bahia 1 (BA1)	15	12	0.96 (0.040)	0.004 (0.001)	13	11	0.96 (0.050)	0.005 (0.001)
	Bahia 2 (BA2)	6	5	0.93 (0.122)	0.004 (0.001)	6	6	1.00 (0.096)	0.003 (0.001)
	Espírito Santo (ES)	10	8	0.96 (0.059)	0.005 (0.001)	10	8	0.96 (0.059)	0.004 (0.001)
	Rio de Janeiro (RJ)	3	2	0.67 (0.314)	0.001 (0.001)	3	2	0.67 (0.314)	0.001 (0.001)
Northwestern Atlantic lineage (NWA)		8	4	0.63 (0.184)	0.003 (0.001)	1	1	–	–
Western Atlantic 1 lineage (WA1)		32	19	0.88 (0.053)	0.003 (0.001)	30	24	0.95 (0.033)	0.003 (0.0004)
Western Atlantic 2 lineage (WA2)		12	6	0.82 (0.096)	0.002 (0.001)	8	7	0.96 (0.077)	0.003 (0.001)
	All samples	52	29	0.94 (0.022)	0.008 (0.001)	39	32	0.97 (0.020)	0.006 (0.001)

π, nucleotide diversity; h, haplotype diversity; n, number of specimens; n_H, number of observed haplotypes.
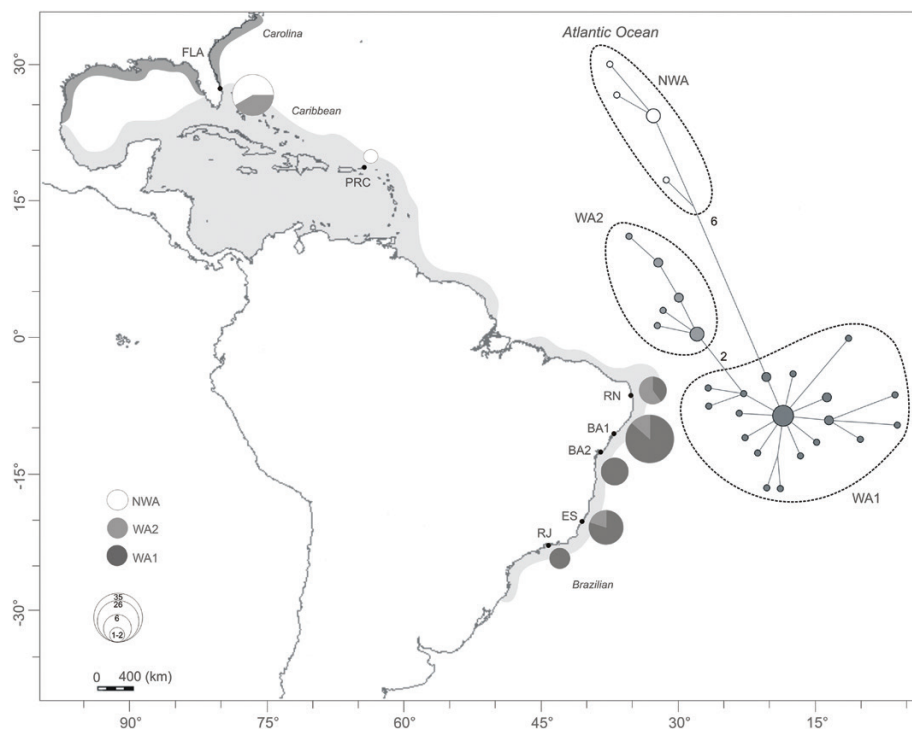

Figure 6. Genealogical relationships among haplotypes of *Bathygobius geminatus* and their geographic distribution based on *COI*. In the parsimony median-joining network, sizes of the circles are proportional to the frequency of each haplotype. Line lengths indicate the number of mutations between haplotypes (shortest lines = 1 mutation). Pie charts in the map represent the ratio of specimens of each lineage at each location (correspondence between circle sizes and number of individuals is indicated in the legend). Colour coding of network and pie charts corresponds to lineages observed: dark grey, WA1 lineage; light grey, WA2 lineage; white, NWA lineage. In the map, the dark grey area corresponds to the warm-temperate biogeographic province (Carolina) and the light grey areas to tropical biogeographic provinces (Caribbean and Brazilian; Briggs & Bowen, 2013).
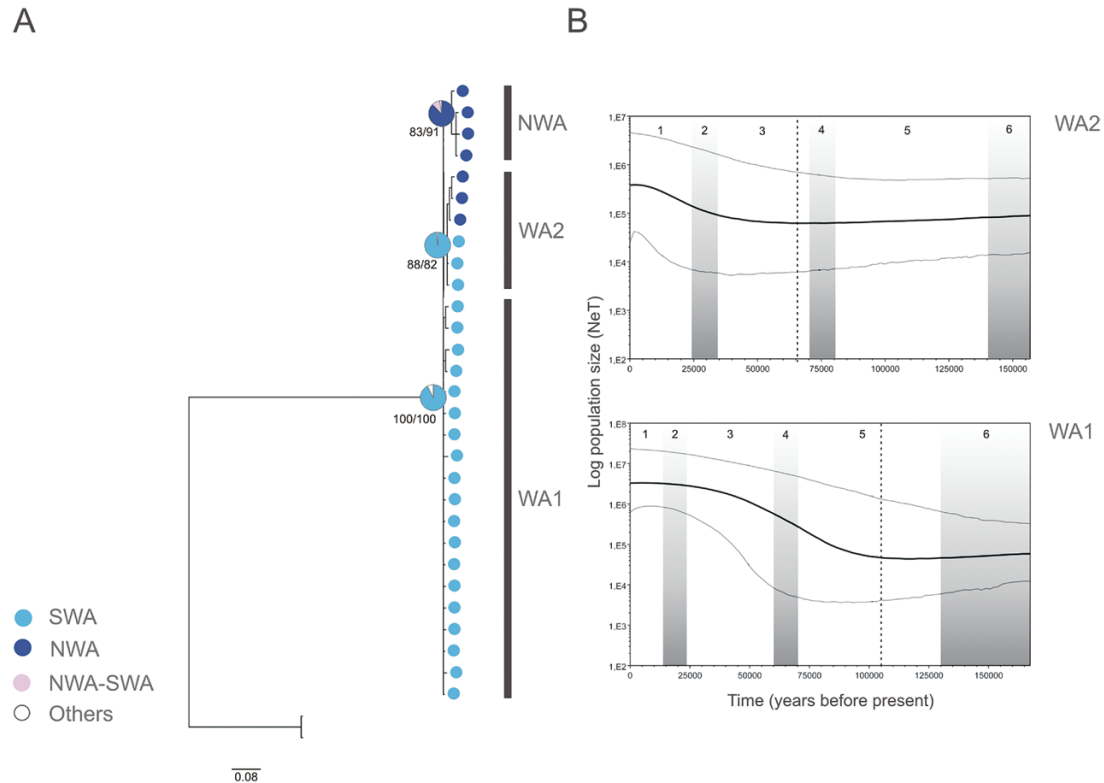
Figure 7. (A) Bayesian phylogenetic tree of haplotypes and (B) BSPs for each lineage of *Bathygobius geminatus* based on *COI*. Bayesian posterior probability values and ML bootstraps are indicated only for the nodes with over 60% support (50% majority-rule consensus tree). In the tree, *B. geminatus* lineages are indicated by vertical bars. Colours denote the distribution as indicated by the embedded key. Current distributions of each haplotype are indicated at terminals. Pie charts of the nodes, with over 75% support for both inference methods, indicate the relative probabilities from ancestral distribution: Southwestern Atlantic (SWA) and Northwestern Atlantic (NWA). For details of the ancestral distributions, see Table 6. In the BSP, the median estimate (black solid line) and 95% HPD limits (grey solid line) are indicated. The maximum time is the upper 95% HPD of the root height. Dotted lines correspond to the approximate onset of the expansion events. The main glaciations over the last 200 thousand years are shaded in grey: MIS6 (190–130 ka), MIS4 (70–60 ka) and MIS2 (24–14 ka; Cohen & Gibbard, 2011).

Table 6. Ancestral distribution reconstructions of *Bathygobius soporator* and *Bathygobius geminatus*

Species/node	Probabilities for nodal reconstructions	
	COI	Cyt *b*
Bathygobius soporator		
WA1	SWA: 0.93; SWA/NWA: 0.07	SWA: 1.00
WA2	–	SWA: 1.00
EA	EA: 0.87; EA/NWA: 0.11; others: 0.02	EA: 0.98; others: 0.02
CAL	NWA: 1.00	–
WA1/WA2	SWA: 0.98; others: 0.02	SWA: 1.00; others: 0.01
WA1/WA2/EA/CAL	NWA: 0.99; others: 0.01	NWA: 0.81; SWA: 0.06; EA: 0.06; others: 0.07
Bathygobius geminates		
NWA	NWA: 0.88; NWA/SWA: 0.10; others: 0.02	–
WA1	–	–
WA2	SWA: 0.98; others: 0.02	SWA: 0.93; SWA/NWA: 0.06; others: 0.01
NWA/WA1/WA2	SWA: 0.93; others: 0.07	SWA: 0.93; others: 0.07

EA, Eastern Atlantic; EP, Eastern Pacific; NWA, Northwestern Atlantic; SWA, Southwestern Atlantic.

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π = 0.008; range: 0.001–0.012), and haplotype diversity was high (overall h = 0.94; range: 0.67–0.96; Table 5). For the cyt *b* gene (1082 bp; n = 39), a total of 71 polymorphic sites, distributed among 32 haplotypes, were identified. Levels of cyt *b* gene variation were similar to those observed for *COI* (overall π = 0.006; range: 0.001–0.007; overall h = 0.97; range: 0.67–1.00; Table 5).

Three major mitochondrial lineages were observed (Fig. 6; Supporting Information, Fig. S8B): one in the Northwestern Atlantic (NWA, which had an average K2P distance of 1.7–1.9% for *COI* and 2.5–2.8% for cyt *b* with WA1 and WA2, respectively) and two lineages distributed in sympatry along the Western Atlantic (WA1 and WA2, which differed by 0.8% for *COI* and 0.9% for cyt *b*). The ancestral reconstruction indicated that the ancestral of *B. geminatus* mtDNA lineages was located in the Southwestern Atlantic or more exactly in the Brazilian province (Fig. 7A; Supporting Information, Fig. S9B; Table 6).

A high genetic differentiation was observed within the Atlantic (overall *COI* Φ_{ST}: 0.31, P < 0.05), but hierarchical analyses failed to find any significant groups (Φ_{CT} = 0.49, P > 0.05). This may result from the low statistical power of AMOVA to detect between-group structure when the number of populations or localities per group is small (Fitzpatrick, 2009). In those cases, pairwise F_{ST}, or its analogous pairwise Φ_{ST}, may be statistically more useful to detect population differences (Fitzpatrick, 2009). The main population structure signal detected by pairwise Φ_{ST} analyses of *B. geminatus* was the high differentiation between Florida in the Northwestern Atlantic and the other localities on the Brazilian coast (Supporting Information, Table S9), where the population from Florida mainly presents haplotypes of the NWA lineage (Fig. 6). Likewise, since the localities with n = 1 from the Northwestern Atlantic were excluded from the analyses of cyt *b* gene, no genetic differentiation was observed within the Brazilian populations (overall Φ_{ST}: 0.07, P > 0.05).

DEMOGRAPHIC ANALYSES OF *B. SOPORATOR* AND *B. GEMINATUS*

To understand the events that shaped the formation of mtDNA lineages in both species, demographic analyses were conducted on lineages rather than populations. The Tajima's D, Fu's F_s and the R_2 statistics of *COI* sequences indicated a significant deviation from neutrality in each lineage for both species (P < 0.05; Supporting Information, Table S10). Those deviations could be explained by selection or demographic factors. Population growth or selective sweeps can produce an excess of recent mutations (therefore an excess of rare alleles) that can result in negative values for Tajima's D and Fu's F_s (Ramírez-Soriano *et al.*, 2008). Regions

that have experienced a selective sweep tend to exhibit low levels of synonymous polymorphism (Macpherson *et al.*, 2007). Thus, considering that more than 90% of the nucleotide polymorphisms in the *COI* gene resulted in synonymous substitutions, it is not unreasonable to assume that the observed deviations are more likely because to a population expansion, which was confirmed by the BSPs (Figs 5B and 7B) and mismatch distribution (Supporting Information, Fig. S10) analyses.

The BF (Supporting Information, Table S11) showed that both molecular clock models were equally well supported [Ln (BF) < 1.1; Kass & Raftery, 1995]. Thus, and considering that relaxed clocks should not be used with intraspecific data sets (Drummond & Bouckaert, 2015), only the results of BSPs with a strict clock are shown (Figs 5B and 7B). In *B. soporator*, the WA2 lineage appears to have experienced a demographic expansion that started at *c*. 130 thousand years ago (ka), during an interglacial episode in Late Pleistocene related to the marine isotope stage (MIS) 5, which became more accentuated about *c*. 45 ka during interglacial MIS3 (Cohen & Gibbard, 2011). The WA1 and CAL lineages also appear to have experienced demographic expansions that started at *c*. 80–75 ka during the interglacial MIS5 (Fig. 5B). In *B. geminatus*, the WA1 lineage experienced a demographic expansion that started at *c*. 105 ka during the MIS5 interglacial episode, whereas the demographic expansion of the WA2 lineage began *c*. 55 ka, during the MIS3 interglacial (Fig. 7B).

DISCUSSION

Our study proposes an evolutionary relationship between pairs of Eastern Pacific and Western Atlantic gobies. It also identifies and describes a new endemic species of *Bathygobius* for the Western South Atlantic, *B. brasiliensis* sp. nov., as well as several old mitochondrial lineages and limited gene flow in the gobies *B. soporator* and *B. geminatus*.

BATHYGOBIUS BRASILIENSIS: A NEW ENDEMIC SPECIES

Lima *et al.* (2005) observed the existence of a deep evolutionary divergence between insular and coastal populations of *Bathygobius* in Northeast Brazil. Later, Tornabene *et al.* (2010) examined a museum specimen from Atol das Rocas and suggested that it fits the description of *B. antilliensis*. Recently, cytogenetic analyses indicated that the populations from Fernando de Noronha and Atol das Rocas have notable differences in karyotype macrostructure, chromosome-banding patterns and localization of 18S rDNA and 5S rDNA in comparison to continental populations of *B*.

soporator and *B. geminatus* (Lima-Filho *et al.*, 2012, 2016). Additionally, Lima-Filho *et al.* (2016) found a remarkable genetic differentiation, with the *COI* gene, between specimens from Fernando de Noronha and Atol das Rocas and specimens of the other Atlantic species, suggesting that they might belong to an undescribed species. According to the multigene phylogenetic analyses carried out in this study, the specimens from Fernando de Noronha and Atol das Rocas form a monophyletic clade, which is indeed distinct from *B. soporator*, *B. antilliensis* and any other *Bathygobius* species studied to date (Fig. 1). The same result was obtained when the sequences published by Lima-Filho *et al.* (2016) were added to our *COI* data set (Supporting Information, Fig. S11). Thus, we confirm the hypothesis of Lima-Filho *et al.* (2016) that a new species of *Bathygobius* exists in those Islands. The analysis, here, of a larger data set that includes samples from through the Brazilian coast and the Caribbean indicates that this new species is an Islands endemic, whose sister species is *B. antilliensis*. The genetic distances between those two species (6.3% for *COI* and 7.4% for cyt *b*) are comparable to or greater than those found among other congeneric species pairs of gobies (Taylor & Hellberg, 2005; Baldwin *et al.*, 2009; Neilson & Stepien, 2009; Victor, 2013) and reef fishes (Rocha, 2004; Rocha *et al.*, 2008a; DiBattista *et al.*, 2011). In addition to the genetic differences, the insular *B. brasiliensis* sp. nov. differs from all Atlantic species of the genus by a combination of several morphological and meristic characters (see Comparisons in the description of *B. brasiliensis* sp. nov. above).

Most of the Brazilian marine fauna is derived from the Caribbean, although Brazil also exports some biodiversity back into the Caribbean (Rocha *et al.*, 2008b). There are several Caribbean species that occur in the Brazilian oceanic islands, but not in the adjacent Brazilian continental shoreline [e.g. the puddingwife *Halichoeres radiatus* (Rocha *et al.*, 2005); the smallmouth grunt *Haemulon chrysargyreum* (Rocha, 2003)]. Moreover, many Caribbean reef fish are phylogenetically related to the Eastern Pacific (Floeter *et al.*, 2008). *Bathygobius brasiliensis* sp. nov. occurs in the Brazilian oceanic islands, and its sister species, *B. antilliensis*, occurs in the Caribbean. These two species diverged *c.* 3.03 Mya in the Pliocene, and they are more closely related to the Eastern Pacific species, *B. ramosus*. These three species diverged *c.* 5.91 Mya in the late Miocene and together with *B. lineatus* from Eastern Pacific constitute the '*B. antilliensis* group', which diverged *c.* 9.30 Mya (Fig. 2, Table 2).

During the Miocene, the Atol das Rocas and Fernando de Noronha islands emerged (*c.* 12 Mya; Cordani, 1970; Floeter *et al.*, 2008), and the biogeographical barriers of Panama Isthmus and Amazon discharge began to form. At ~11 Mya, coinciding with global sea-level drop, the major uplift of the Andes and climate cooling, the Andean sediments for the first time reached the Atlantic coast through the Amazon drainage system, marking thus the onset of the Amazon Discharge. Between 7.9 and 6.0 Mya, the intensification of Andean erosion increased the sedimentation rates on the fan, and the Amazon River became fully established at ~7 Mya (Figueiredo *et al.*, 2009; Hoorn *et al.*, 2010). On the other hand, between 23 and 25 Mya, the collision of the Panama Block and South America began, marking the onset of the formation of Panama Isthmus. At ~10 Mya, the exchange of deep and intermediate waters between the Caribbean and the Pacific ended, and at ~3.5 Mya during the Pliocene, occurred the final closure of Panama Isthmus ending the exchange of shallow waters (Coates & Obando, 1996; Farris *et al.*, 2011; Bacon *et al.*, 2015; Montes *et al.*, 2015). The final closing of the Panama Isthmus caused marked changes in the Atlantic circulation during the Pleiocene, which produced instability due to hydrographic imbalance (Haug & Tiedemann, 1998). Later, sea-level fluctuations during the glacial–interglacial cycles in the Pleistocene modified the configuration of the Amazon discharge, resulting in sporadic periods of reduced gene flow that, in some cases, could lead to speciation, albeit not as effectively as hard barriers such as the IOP (Rocha, 2003; Moura *et al.*, 2016).

Considering the geological and hydrographic history of the Atlantic basin and our results, we propose a hypothetical scenario of dispersal and vicariance about the evolutionary history of the species of *B. antilliensis* group. The most ancient speciation event (*B. lineatus*–*B. ramosus*/*B. brasiliensis*/*B. antilliensis*) occurred in the Eastern Pacific during the Miocene (*c.* 9 Mya). *Bathygobius lineatus* is found in oceanic islands of Eastern Pacific, and *B. ramosus* can be found along the Eastern Pacific coast, from the Gulf of California in Mexico to northern Peru (Van Tassell, 2011). Those distributions suggest that a plausible speciation scenario involved a dispersal event from the western American coast, where there was an ancestral population established, to oceanic islands of the Eastern Pacific. Thus, the colonization of the new oceanic islands possibly drove the speciation event that resulted to the divergence of *B. lineatus* and the ancestor to *B. ramosus*/*B. brasiliensis*/*B. antilliensis*. Subsequently, in the evolutionary history of *B. antilliensis* group, there was an event of dispersal and vicariance between the Atlantic and Pacific oceans. The Eastern Pacific ancestor colonized the Northwestern Atlantic prior to the final closure of the Panama Isthmus, leading to the establishment of an ancestral population in the Eastern Pacific and Northern Atlantic. Once that ancestral population was established, the interruption of the marine connection between the Caribbean and the Pacific by the emergence of the isthmus possibly led

to the divergence of *B. ramosus* and the ancestor to *B. antilliensis*/*B. brasiliensis* (*c*. 6 Mya). These results are in concordance with those from other studies, which indicate that many marine clades exhibit dispersal/vicariance pulses between the Atlantic and Pacific oceans that took place between *c*. 8 and 6 Mya (Bacon *et al.*, 2015). In the Pliocene, after the closure of the Panama Isthmus, the most recent speciation event (*B. antilliensis*–*B. brasiliensis* sp. nov., *c*. 3 Mya) occurred. During that period, changes in the Atlantic circulation by the final closure of the isthmus and the intermittent nature of the Amazon mouth barrier may have promoted the colonization of the Southwestern Atlantic by the population from the Northwestern Atlantic and related speciation event (Figs 1, 2).

REMARKS ABOUT THE DISTRIBUTION OF *BATHYGOBIUS* SPECIES

To date, the complete distributions of *B. antilliensis* and *B. geminatus* remain unclear. Tornabene *et al.* (2010) indicated that *B. antilliensis* might occur off the continental Colombian coast because the illustration of Colombian '*B. soporator*' in Garzón-Ferreira & Acero (1992) appears to be this species. Our results confirm this observation, given that the samples from the island of the Colombian Caribbean (San Andrés) are *B. antilliensis*, as are the specimens from Barbados. On the other hand, the sample from the Colombian continental coast (Cartagena) is *B. soporator*. Tornabene *et al.* (2010) suggested that *B. geminatus* could occur in Brazil based on the analysis of a photograph of a Brazilian specimen identified as *B. mystacium* on FishBase. Recently, Lima-Filho *et al.* (2016) confirmed that *B. geminatus* occurs in the waters of Rio Grande do Norte State. Here, we found that *B. geminatus* has a much wider distribution than previously thought, occurring in sympatry along the continental coast of Brazil with *B. soporator* (Supporting Information, Fig. S1). Our analyses also indicate that *B. mystacium*, which is morphologically similar to *B. geminatus*, probably does not occur in Brazil, since none of the 146 individuals collected along ~5000 km of the Brazilian coast belonged to that species. This is important because many authors have considered *B. mystacium* as a common goby species in Brazil (Floeter *et al.*, 2003; Macieira & Joyeux, 2011), even in localities where all specimens analysed by us were found to be *B. geminatus* or *B. soporator*. Therefore, we recommend that identifications of *B. mystacium* in Brazil be verified.

PHYLOGEOGRAPHY OF *B. SOPORATOR* AND *B. GEMINATUS*

The phylogeographic analyses revealed a similar pattern of genetic diversity for both *B. soporator* and *B.* *geminatus*. Both species presented deeply differentiated lineages (CAL and EA lineages for *B. soporator* and NWA lineage for *B. geminatus*), separated from other related lineages (WA lineages) by 6–13 and 25–34 mutations for *COI* and cyt *b*, respectively (Figs 4, 6; Supporting Information, Fig. S8). This deep divergence could indicate a relatively old partition corresponding to the major biogeographic provinces. In some reef fishes and sea urchins, for example, the deep genetic divergences observed among Atlantic biogeographic provinces (d = 2.3–12.7% for cyt *b*) were attributed to the presence of cryptic species within the Atlantic (Muss *et al.*, 2001; Carlin *et al.*, 2003; Lessios *et al.*, 2003; Rocha, 2004). The divergence values of the most differentiated lineages of *B. soporator* (median d = 2.7% for *COI*; median d = 4.1% for cyt *b*) and *B. geminatus* (median d = 1.8% for *COI*; median d = 2.7% for cyt *b*) are similar to those observed between cryptic species of some reef fishes, but are smaller than those found between sister species in the genus *Bathygobius* (Table 1). Because of the lack of diagnostic morphological or pigmentation characters (Tornabene *et al.*, 2010), and the monomorphism in the nuclear genes *RAG1* (Tornabene & Pezold, 2011) and Rhodopsin (data not shown), we conservatively chose not to assign those lineages to distinct species. Genetic divergence without obvious morphological differences has been observed in the wrasse *Halichoeres bivittatus* between the tropical and subtropical habitats (d = 3.4% for cyt *b*; Rocha *et al.*, 2005).

The two most closely related lineages (WA1 and WA2) of *B. soporator* and *B. geminatus* were also observed in sympatry throughout the tropical Western Atlantic and were separated by two *COI* and five to seven cyt *b* mutations, indicating a recent divergence (Figs 4, 6; Supporting Information, Fig. S8). Co-existence of different mitochondrial lineages in sympatry has also been observed in reef fishes such as the surgeonfish *Naso brevirostris* (Horne *et al.*, 2008), the coral trout *Plectropomus maculatus* and the snapper *Lutjanus carponotatus* (Evans *et al.*, 2010). In each WA lineage of *B. soporator*, the Caribbean and Brazilian provinces shared the most common haplotype and four other central haplotypes. The central position of shared haplotypes indicates an old age for the connections between provinces. In *B. geminatus*, our results suggest that the lineages co-occur in the Brazilian coast and that the WA1 lineage may be restricted to the Brazilian province. However, because of the small sample sizes in the Caribbean, we cannot affirm that the WA1 lineage really does not occur in the Caribbean, as observed for the WA lineages of *B. soporator*.

Two primary hypotheses have been proposed to explain the high biodiversity of regions such as the Greater Caribbean: the centre of origin (CO) hypothesis and the centre of accumulation (CA) hypothesis

(Rocha *et al.*, 2008b). The CO hypothesis proposes that species originate in the centre and disperse to the periphery, whereas the CA hypothesis proposes that diversity centres accumulate species that originated elsewhere (Rocha *et al.*, 2008b; Bowen *et al.*, 2013). Therefore, under a CO hypothesis, the ancestral haplotypes should be found at the centre of diversity, and under a CA hypothesis, the ancestral haplotypes should be found in peripheral populations. In *B. soporator*, the ancestral haplotype was distributed in the Northwestern Atlantic (Fig. 5A; Supporting Information, Fig. S9A), indicating a Greater Caribbean ancestor for the lineages that dispersed towards the Southern Atlantic, supporting the CO hypothesis. In contrast, the ancestral distribution of *B. geminatus* was in the Southwestern Atlantic (Fig. 7A; Supporting Information, Fig. S9B), indicating that the lineages probably derived from a Brazilian ancestor that dispersed towards the Northern Atlantic, supporting the CA hypothesis. Genetic patterns that support both or either of the hypotheses for the Greater Caribbean have also been reported in other reef fishes (e.g. Rocha *et al.*, 2008b; Castellanos-Gell *et al.*, 2012), indicating that these two models are not mutually exclusive, but instead may operate in concert.

Recent phylogeographic and demographic studies have shown that climatic fluctuations during the Pleistocene (*c*. 2.6 Mya to 10 ka) influenced the demographic history and distribution of several marine species (e.g. Bowen *et al.*, 2006; Rodríguez-Rey *et al.*, 2013). During those periods, coral reef habitats experienced massive fluctuations in distribution and quality because of changes in sea level and temperature (Daly, 1915; Kiessling *et al.*, 2012). Reef habitats may have been reduced by 90% in the Caribbean during the glacial periods, when the sea level was at least 100 m below current levels, decreasing the available habitats for reef fishes and the connectivity among populations (Bellwood & Wainwright, 2002; Bowen *et al.*, 2006). During interglacial periods, the rising sea level increased habitat availability, resulting in population expansions of marine species (Bowen *et al.*, 2006). Our results indicate that the expansions observed in the lineages of both species of *Bathygobius* could have occurred during interglacial periods in Late Pleistocene (MIS5 and MIS3; Figs 5B and 7B). Thus, during those interglacial periods, the sea-level rise may have weakened the Amazon barrier (see the discussion below), increasing habitat availability and allowing the dispersal from Caribbean to Brazil for *B. soporator* and from Brazil to the Caribbean for *B. geminatus*. Demographic expansion events have also been detected during interglacial periods in several reef fishes in the Atlantic [e.g. in the squirrelfishes *Myripristis jacobus* and *Holocentrus ascensionis* (Bowen *et al.*, 2006); in

the sand goby *Pomatoschistus minutes* (Gysels *et al.*, 2004; Larmuseau *et al.*, 2009)].

POPULATION STRUCTURE OF *B. SOPORATOR* AND *B. GEMINATUS*

The pattern of genetic structuring found in *B. soporator* and *B. geminatus* suggests a restricted gene flow among the major biogeographic provinces. In *B. soporator*, a high population genetic differentiation was observed between three groups: (1) Guinea in the Tropical Eastern Atlantic province, (2) the Carolina province and (3) the tropical Western Atlantic (Caribbean + Brazilian provinces, which also showed some differentiation). In *B. geminatus*, a high differentiation was observed between the localities in the Northwest (Carolina + Caribbean provinces) and Southwest Atlantic (Brazilian province; Supporting Information, Tables S6–S8).

The divergence observed in *B. soporator* between the Eastern Atlantic (Gulf of Guinea) and Western Atlantic (Carolina + Caribbean + Brazilian provinces) can be attributed to the thousands of kilometres of blue water distances (mid-Atlantic Barrier) and the biological traits that determine the dispersal potential of species. *B. soporator* is a sedentary benthic species with adhesive demersal eggs, nest-guarding by the male, and its larvae can live in the plankton for up to 26–31 days (Tavolga, 1954; Peters, 1983). Scheltema (1971) estimated that the distance from Gulf of Guinea to Brazil through the South Equatorial Current could be passively traversed in 60–154 days and that the distance from Brazilian coast to São Tomé through the Equatorial Undercurrent could be crossed in 96 days. The mid-Atlantic barrier is considered a soft filter, whose effectiveness could have been influenced by ocean-currents dynamics that varied with the fluctuations in the sea levels over the past 10 Myr (Floeter *et al.*, 2008). It is possible that those events allowed occasional crossings that led to the establishment of the Eastern Atlantic lineage even though the current dynamics acts as a barrier to dispersal, isolating the populations in the Eastern and Western Atlantic, as inferred in other marine species [e.g. in the damselfish *Chromis multilineata* (Rocha *et al.*, 2008b), in the squirrelfish *H. ascensionis* (Bowen *et al.*, 2006) and in the urchin *Tripneustes ventricosus* (Lessios *et al.*, 2003)].

The high genetic differentiation observed between populations in the Carolina province and in the tropical Western Atlantic (Caribbean and Brazilian provinces) in *B. soporator* could be related to many factors. The Carolina province has subtropical characteristics as warm-temperate waters, while the Caribbean and the Brazilian provinces have tropical characteristics. The

CAL and WA lineages overlap in SE Florida, similar to what is found in *H. bivittatus* (Rocha *et al.*, 2005). The SE Florida area is considered a mix of overlap between tropical and subtropical faunas (Robertson & Cramer, 2014), and the occurrence of the two *B. soporator* lineages in that area is, thus, not surprising. Ecological and hydrological differences between the areas could also be playing a role in the genetic differentiation of *B. soporator* from those two areas, but this can be challenged by the fact that this species lives in tidal pools that vary strongly in temperature and salinity even during a single day. Their eurybiosis makes it hard to attribute differentiation between areas to environmental factors alone.

Several studies have revealed evolutionary partitions that correspond to ecological discontinuities, particularly the Amazon barrier, between tropical reef habitats of Caribbean and Brazilian provinces (Rocha *et al.*, 2002; Rocha, 2003, 2004). Underneath the Amazon river plume, there is a reef system of ~9500 km² that extends from the Brazil–French Guiana border to Maranhão State, Brazil (Collette & Rützler, 1977; Rocha, 2003; Moura *et al.*, 2016). In this system, there is an assemblage of reef-associated organisms typical of the Western Atlantic mesophotic, with reef structures and rhodolith beds in depths ranging from 30 to 120 m (Moura *et al.*, 2016). Although the juveniles or adults of shallow-water species, as *Bathygobius*, are not able to use this reef system as a stepping-stone between the Brazilian and Caribbean provinces because the system is in relatively deep areas, this corridor probably can be used by larvae to cross the Amazon barrier (Rocha, 2003; Luiz *et al.*, 2012; Moura *et al.*, 2016). Furthermore, the physical configuration of the Amazon barrier fluctuated during the Pleistocene glacial–interglacial cycles, providing an intermittent connectivity matrix between the Caribbean and Brazilian provinces (Rocha, 2003; Moura *et al.*, 2016). On the other hand, ecological traits as large latitudinal-range can facilitate the establishment of species across the Amazon barrier (Luiz *et al.*, 2012). Latitudinal range may be associated with the degree of tolerance to varying environmental conditions by both demersal adults and pelagic larvae, so the eurytolerance or the multiple-habitat use by adults and larvae may facilitate expansion through a stepping-stone effect (Luiz *et al.*, 2012). Thus, the intermittent nature of the Amazon barrier can affect the population structure of reef fishes in different ways (Rocha, 2003; Floeter *et al.*, 2008; Luiz *et al.*, 2013). For example, the freshwater outflow of the Amazon River strongly affects the population structure of *Acanthurus bahianus*, *H. ascensionis* and the sea urchin *T. ventricosus*; moderately affects that of *Acanthurus coeruleus* and *Halichoeres poeyi*; and has no effect on connectivity of *Acanthurus chirurgus* and of *M. jacobus* (Rocha *et al.*, 2002, 2005; Lessios *et al.*,

2003; Bowen *et al.*, 2006). According to Floeter *et al.* (2008), demersal-spawning and brooder families with small-bodied cryptobenthic species (e.g. Chaenopsidae, Gobiidae) seem to be most affected by the Amazon freshwater and sediment discharge. Additionally, the habitat eurytolerance of *B. soporator* and probably of *B. geminatus* may facilitate the establishment of these species once they cross the Amazon barrier (Luiz *et al.*, 2012). Ours results suggest that the population structure of *B. geminatus* could be more affected by the intermittent characteristics of that barrier than that of *B. soporator*. Nevertheless, genetic data from larger population samples of both species from Caribbean will be needed to better estimate levels of gene flow between these two biogeographic provinces.

In conclusion, our study shows the important role of Atlantic biogeographical barriers in shaping the genetic diversity and genetic structure of *Bathygobius* species. The changes in the Atlantic circulation by the final closure of the isthmus and the intermittent nature of the Amazon mouth barrier could have influenced the formation of the pair of sister species, *B. brasiliensis* sp. nov. and *B. antilliensis*. Likewise, the presence of distinct mitochondrial lineages in *B. soporator* and *B. geminatus* indicates a relatively old partition, corresponding to the major biogeographic provinces. Finally, the population structure observed in both species seems to have been determined by the intermittent nature of biogeographical barriers (especially the Amazon discharge and the mid-Atlantic barrier), ecological and biological traits of species, and ecological and hydrological characteristics of those regions.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Appendix S1. Supplementary tables.
Appendix S2. Supplementary figures.
Appendix S3. Material examined.