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Genetic detection of cryptic species in the frillfin goby *Bathygobius soporator*

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Abstract

We compared, through allozyme and cytochrome *b* sequence analyses, populations of *Bathygobius soporator* from four localities on the Brazilian coast with those from the Oceanic Islands of the Rocas Atoll (230 km off the Brazilian coast) and the Bahamas (5700 km northwest from Rocas). The population from the Rocas Atoll was genetically more similar to the geographically distant Bahamas (gene identity, I=0.92) than to the supposedly conspecific populations from the Brazilian coast (I<0.55). Five diagnostic allozyme *loci* and a high level of nucleotide divergence (Kimura 2-parameters=0.21) were found between the two groups, further confirming that they must belong to different biological species. Since the type locality of *B. soporator* is in the Caribbean, the binomial should be maintained for the Bahamas/Rocas Atoll populations, and the other Brazilian populations of *Bathygobius* analysed either represent the occurrence of a species of *Bathygobius* hitherto not cited for the Brazilian coast, or belong to a new species. The coastal populations of this species were also found to be highly structured (F_{ST}=0.18; p<0.05), indicating that, even along the coast, levels of gene flow of this species are limited. This could be related to their reproductive biology (adhesive benthic eggs with parental care and short length of larval life). The timings of the divergence between the Caribbean and the Southwest Atlantic species found here, estimated from allozymes and cytb sequences, are very similar (around 10 MY bp), and correlate with the start of the Amazon river outflow. The colonization of the Rocas Atoll by populations from the Caribbean seems to have taken place after the Caribbean and the Brazilian coast species had diverged.

Keywords: Allozymes; Atlantic Ocean; Biogeography; Cytochrome *b*; Fish; Mitochondrial

1. Introduction

The frillfin goby *Bathygobius soporator* (Valenciennes, 1837) is a bottom-dwelling species that lives in

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tidal pools on coastal regions and oceanic islands on both sides of the Atlantic. In the West Atlantic, it occurs from the Florida Keys (Caribbean) to Santa Catarina (South Brazil) (Cervigón, 1966; Miller and Smith, 1989). In the East Atlantic, *B. soporator* has been reported from the African coast from Senegal to Angola (Cervigón, 1966; Miller and Smith, 1989; Afonso et al., 1999). Four other species of this genus are found in the Atlantic Ocean: *Bathygobius curacao* and *Bathygobius mystacium* are endemic of the West Atlantic, and *Bathygobius casamancus* and *Bathygobius burtoni* are endemic of West Africa (Bohlke and Chaplin, 1993; Brito and Miller, 2001). In Brazil, *B. soporator* occurs along most of the coast, from the north and northeast to the south (1°S to 26° S), whereas *B. mystacium* is limited to the northeastern region (3°S to 18°S) (Miller and Smith, 1989; Bohlke and Chaplin, 1993).

Owing to their bottom-dwelling habits, several morphological characteristics, such as the body lateral line and some bones, are reduced or absent in some species of the Suborder Gobioidei. Thus, it is difficult to assess the biological diversity of gobiid fishes based only on morphological characters (Akihito et al., 2000). Many marine species that were considered to have large geographical distributions were found to be, under close scrutiny, complexes of morphologically similar, but genetically distinct species (review in Knowlton, 2000). Hence, the supposed amphiatlantic distribution of a species with a 'low morphology' (sensu Van Oppen et al., 1997) and with very limited dispersal capabilities (adhesive eggs and short larval pelagic period, Tavolga, 1953; Peters, 1983) is likely to be a taxonomical artifact.

Molecular methods are suitable to detect sibling species in marine organisms (for reviews see, e.g. Knowlton, 1993; Thorpe and Solé-Cava, 1994; Knowlton, 2000). Hence, their use has become common in the recognition of species boundaries and phylogenetic patterns in gobioid fishes (e.g. McKay, 1993; Miller et al., 1994; Akihito et al., 2000; Chen et al., 2002; Gysels et al., 2004). In this study we used allozymes and cytochrome *b* sequences to compare populations from six West Atlantic localities: four along the Brazilian coast and two from the oceanic islands of the Rocas Atoll (250 km off the Brazilian coast) and the Bahamas. The results clearly show the existence of two different species within what was originally identified as *B. soporator*.

2. Materials and methods

2.1. Sample collections

Ninety one samples of *B. soporator* were collected from five localities in Brazil and one locality in the Bahamas (Fig. 1). The Bahamas (Lee Stocking Island—Lab beach) and Rocas Atoll (Farol Island—Ruínas do Farol beach) represent two oceanic islands on the Caribbean and Brazilian coasts, respectively. The Brazilian continental coast localities were Ceará (Fortaleza—Mucuripe beach), Rio Grande do Norte (Natal—Artistas beach), Paraíba (João Pessoa—Cabo Branco beach) and Rio de Janeiro (Mangaratiba—Junqueira beach). Fish identification followed Bohlke and Chaplin (1993). All specimens collected had the morphological characters that are considered diagnostic of *B. soporator* (18–20 pectoral fin rays, 37–41 lateral scales, 0.12–0.14 maxilla length/standard length; Table 1) and different from those of *B. mystacium, B. curacao, B. burtoni* and *B. casamancus*, the other accepted species of *Bathygobius* in the Atlantic. The 17 fish morphologically analysed were deposited at the Museu Nacional do Rio de Janeiro (MNRJ 27704–27715).

2.2. Allozyme electrophoresis

We analysed fragments of muscle tissue from *B. soporator* from the six populations using 12.5% starch-gel allozyme electrophoresis as previously described (Solé-Cava and Thorpe, 1986; Klautau et al., 1999), using a 0.25 M Tris, 0.01 M EDTA, 0.06 M citrate, pH 8.0 (TC8) buffer system (Ward and Beardmore, 1977). Enzyme stain recipes were from Manchenko (1994). We tested 17 enzyme systems, from which we chose 13, based on their consistent and reproducible results: Adenilate kinase (AK, Enzyme Commission #2.7.4.3), alcohol dehydrogenase (ADH, EC #1.1.1.1), alpha esterases (α-EST, EC #3.1.1.1), glutamate dehydrogenase (GDH, EC #1.1.1.49), isocitrate dehydrogenase (IDH, EC #1.1.1.42), lactate dehydrogenase (LDH, EC #1.1.1.27), malate dehy-

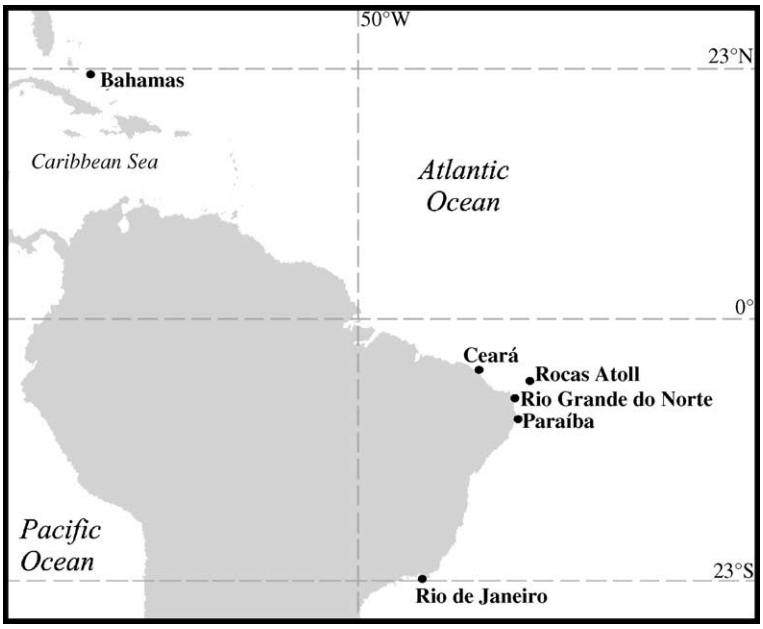

Fig. 1. Sampling sites.

drogenase (MDH, EC #1.1.1.37), malic enzyme (ME, EC #1.1.1.40), mannose 6-phosphate isomerase (MPI, EC #5.3.1.8), phosphogluconate dehydrogenase (PGD, EC #1.1.1.44), phosphoglucose isomerase (PGI, EC #5.3.1.9), phosphoglucomutase (PGM, EC #5.4.2.2) and superoxide dismutase (SOD, EC #1.15.1.1).

2.3. Cytochrome b gene sequencing

We isolated genomic DNA from muscle tissue from seven specimens, by proteinase digestion in lysis buffer (0.1 M Tris, pH 8.0, 0.05 M EDTA, 0.2 M NaCl, 1% w/v of SDS and 3 mg of proteinase K) followed by extraction with the phenol/chloroform

Table 1
Meristic variation and body proportions of *B. soporator* (BS), *B. mystacium* (BM), *B. curacao* (BCu), *B. burtoni* (BB) and *B. casamancus* (BCs)

	Species	BS	BM	BCu	BB	BCs
Fin rays						
Pectoral	18–20[a]	(18–21[b;d]; 19–21[c])	18–20[b]; 19–20[c]	15–18[b]; 16–17[c]	18–20[d]	17–19[d]
1st dorsal	VI–VII[a]	(=VI[b;c;d])	–	–	VI–VII[d]	VI[d]
2nd dorsal	I/7–10[a]	(<20[b;c]; I/7–9[d])	–	–	I/9–10[d]	I/10–11[d]
Ventral	I/5+I/5[a]	(I/5+I/5[c;d])	–	–	I/5+I/5[d]	I/5+I/5[d]
Anal	I/7–10[a]	(I/7–10[d])	–	–	I/9[d]	I/9[d]
Caudal	12–19[a]	(14–15[d])	–	–	14–15[d]	14–15[d]
Pre-dorsal scales	22–24[a]	(17–27[d])	–	–	13–18[d]	14–21[d]
Lateral scales	37–41[a]	(37–41[b;c]; 33–40[d])	33–36[b;c]	31–34[b;c]	33–38[d]	34–39[d]
HL/SL (%)	25–33[a]	(27–31[d])	–	–	15–31[d]	24–30[d]
HW/SL (%)	17–28[a]	(16–18[d])	–	–	14–17[d]	13–17[d]
ML/SL (%)	12–14[a]	(12–16[b];10–14[d])	9–12[b]	11–14[b]	9–11[d]	8–9[d]
PF/SL (%)	22–33[a]	(21–23[d])	–	–	21–26[d]	19–26[d]
VF/SL (%)	18–23[a]	(19–23[d])	–	–	21–24[d]	18–24[d]

References: a—This work; b—Bohlke and Chaplin (1993); c—Murdy and Hoese (2004); d—Miller and Smith (1989).
HL=head length; HW=head width; ML=maxilla length; PF=pectoral fin length; VF=ventral fin length; SL=standard length.

method, as described in Sambrook et al. (1989). The extracted DNA was precipitated with ethanol and ammonium acetate, resuspended in 50 μL of ultrapure water and stored at −20 °C.

The amplification reactions of cytochrome *b* (cytb) were done using the primers CytB2F [5′-GTG ACT TGA AAA ACC ACC GTT G] (Song et al., 1998) and CytB2R [5′-AAT AGG AAG TAT CAT TCG GGT TTG ATG] (Taberlet, 1992). The sequencing reactions were done using the internal primers L15287 [5′-GTA ATT CAA CTA CAA GAA C] (Briolay et al., 1998) and BathyR [5′-GGT AAA GTT TTC TGG GTC TCC] (designed from aligned cytochrome *b* sequences of several gobiid species obtained from GenBank). The PCR reactions were made with 1 unit of Taq polymerase (Invitrogen), 200 μM of each dNTP, 1.5 mM of $MgCl_2$, 0.5 μM of each primer, 50 μg of acetylated bovine serum albumin in 25 μL of PCR buffer (Invitrogen). We used the following thermocycling conditions: one initial cycle of 5 min at 94 °C, 1 min at 61 °C and 2 min at 72 °C, followed by 35 cycles of 1 min at 94 °C, 1 min at 61 °C and 1 min at 72 °C, and a final extension of 5 min at 72 °C. A negative control (without template DNA) was used in all PCR reactions. The PCR products were purified with the Exonuclease I/Shrimp alkaline phosphatase method (ExoSap, USB, Columbus, OH, USA) and were sequenced through the dideoxi method, on an ABI 3700 automatic sequencer. The sequences analysed were deposited in GenBank with accession numbers AY781105–AY781111.

2.4. Data analyses

2.4.1. Allozymes

We estimated gene frequencies, heterozygosities, fits to Hardy–Weinberg equilibrium and inbreeding indices (F_{IS}, F_{ST}; Wright, 1978) from allozyme genotype proportions, using the GENETIX program version 4.02 (Belkhir et al., 1996). The same program was also used to test the 95% confidence limits of F_{IS}, F_{ST} and linkage disequilibrium using 1000 Monte Carlo permutations. The genetic similarity between each pair of populations was estimated using Nei's (1978) unbiased indices of genetic identity and distance, using the BIOSYS program version 1.7 (Swofford and Selander, 1981). Genetic Identity data were used to construct a UPGMA tree (Sneath and Sokal, 1973). This tree construction method was chosen for the allozyme data because it has been shown to give better estimates of tree topology when the variance is large, as in the case of distance estimates based on gene frequencies with small samples (Nei et al., 1983). The statistical significance of all tests was corrected using a Bonferroni sequential analysis (Lessios, 1992).

2.4.2. Cytochrome b

The cytochrome *b* sequences were aligned using the Clustal W program (Higgins et al., 1994). No indels were observed. The alignments were used to estimate the nucleotide divergences between sequences using the Kimura 2-parameter distance (Kimura, 1980) with Gamma correction (γ=0.298), from which a neighbor-joining tree (Saitou and Nei, 1987) with 1000 bootstrap replicates was built, using the MEGA 2.1 software (Kumar et al., 2001). The Gamma parameter was estimated using the GZ-Gamma program (Gu and Zhang, 1997).

For both allozyme and cytb data an analysis of molecular variance (AMOVA; Excoffier et al., 1992) was done using the Arlequin 2000 program (Schneider et al., 2000). The groups compared were defined ad hoc, from the trees produced from allozyme and cytb data.

3. Results

The 13 enzymes selected coded for 21 *loci* (*ADH**, *AK**, *α-EST-1**, *α-EST-2**, *GDH**, *IDH**, *LDH-1**, *LDH-2**, *MDH-1**, *MDH-2**, *ME-1**, *ME-2**, *MPI**, *PGD-1**, *PGD-2**, *PGI-1**, *PGI-2**, *PGM-1**, *PGM-2**, *PGM-3** and *SOD**) (Table 2). We did not observe any linkage disequilibrium nor departures from Hardy–Weinberg expectations (Fisher exact test; p>0.05, after Bonferroni correction; Lessios, 1992) at any *locus* studied. The average heterozygosity values ranged from 0.031±0.020 (Rio Grande do Norte) to 0.071±0.043 (Rocas Atoll), all within the typical range of heterozygosity values reported for allozymes in marine fishes (Ward et al., 1994). The populations of Bahamas/Rocas Atoll could be readily distinguished from the continental Brazilian populations by the presence of five diagnostic *loci* (*AK**, *α-EST-2**, *GDH**, *MPI** and

Table 2
Gene frequencies for the six populations of *Bathygobius soporator* analysed

Locus	Bahamas	Rocas Atoll	Ceará	Rio Grande do Norte	Paraíba	Rio de Janeiro
*AK**						
(*N*)	19	4	15	10	17	4
1	1.000	1.000	0	0	0	0
2	0	0	1.000	1.000	1.000	1.000
*α-EST-1**						
(*N*)	13	3	18	11	27	4
1	0	0	0.528	0.045	0	0
2	0	0	0.389	0.909	0.630	0.375
3	0.308	0	0.056	0	0.074	0.625
4	0	0	0.028	0.045	0.296	0
5	0.692	1.000	0	0	0	0
*α-EST-2**						
(*N*)	12	3	9	4	5	3
1	0	0	1.000	1.000	1.000	1.000
2	0.958	1.000	0	0	0	0
3	0.042	0	0	0	0	0
*GDH**						
(*N*)	17	4	16	13	17	4
1	1.000	1.000	0	0	0	0
2	0	0	1.000	1.000	1.000	1.000
*ME-2**						
(*N*)	19	4	21	9	24	3
1	0.921	0	0	0	0	0
2	0.079	1.000	0	0	0.042	0
3	0	0	1.000	1.000	0.958	1.000
*MPI**						
(*N*)	9	4	12	8	12	4
1	1.000	1.000	0	0	0	0
2	0	0	1.000	1.000	1.000	1.000
*PGD-1**						
(*N*)	19	4	20	16	24	4
1	0.158	0	0.050	0	0	0.125
2	0.842	1.000	0.150	0	0	0
3	0	0	0.800	1.000	1.000	0.875
*PGI-1**						
(*N*)	17	4	14	10	18	4
1	0	0	0.179	0	0	0
2	0	0	0.750	0.600	0.917	0.750
3	0.882	0.500	0.071	0.350	0.083	0.250
4	0.118	0	0	0.050	0	0
5	0	0.500	0	0	0	0
*PGI-2**						
(*N*)	18	4	15	12	18	4
1	0	0	0.233	0.083	0	0

(continued on next page)

Table 2 (*continued*)

Locus	Bahamas	Rocas Atoll	Ceará	Rio Grande do Norte	Paraíba	Rio de Janeiro
*PGI-2**						
2	0.833	0.875	0.767	0.917	1.000	1.000
3	0.167	0.125	0	0	0	0
*PGM-1**						
(*N*)	9	4	12	8	11	2
1	0	0.250	0	0	0	0
2	0	0.625	0.167	0	0	0
3	0.222	0.125	0.417	0.375	0.318	1.000
4	0.056	0	0.167	0.250	0.318	0
5	0.611	0	0.208	0.313	0.318	0
6	0.111	0	0.042	0.063	0.045	0
*PGM-2**						
(*N*)	9	4	8	9	12	3
1	0.222	0	0.625	0.556	0.667	1.000
2	0.778	1.000	0.375	0.444	0.333	0
*PGM-3**						
(*N*)	18	4	20	15	18	3
1	0.972	1.000	1.000	1.000	1.000	1.000
2	0.028	0	0	0	0	0
*SOD**						
(*N*)	7	4	5	4	4	0
1	0	0	1.000	1.000	1.000	0
2	1.000	1.000	0	0	0	0
H_{OBS}	0.059	0.071	0.054	0.031	0.038	0.048
H_{EXP}	0.177	0.058	0.141	0.102	0.093	0.058

The loci *ADH**, *IDH**, *LDH-1**, *LDH-2**, *ME-1**, *MDH-1**, *MDH-2** and *PGD-2** were fixed for the same allele in all populations. *N*: number of individuals analysed; H_{OBS}: observed (direct count) heterozygosity; H_{EXP}: Hardy–Weinberg expected heterozygosity.

*SOD**; Table 2), resulting in low levels of genetic identity between the two groups ($0.558<I<0.665$) (Table 3). Genetic identities were high between the populations from Bahamas and Rocas Atoll ($I=0.921$), and among the populations along the Brazilian coast ($0.963<I<0.995$; Table 3 and Fig. 2). Nevertheless, the populations were genetically structured both along the Brazilian coast ($0.013<F_{ST}<0.183$; $p<0.05$) and between the Bahamas and Rocas Atoll ($F_{ST}=0.370$; $p<0.05$) groups.

We found 118 parsimony informative sites (out of 671 nucleotides sequenced) in the seven sequences obtained from *B. soporator* from Bahamas, Rocas Atoll, Rio Grande do Norte and Rio de Janeiro (Fig.

Table 3
Unbiased genetic distances (below diagonal) and identities (above diagonal) (Nei, 1978) between the six populations of *Bathygobius soporator* analysed

Population	1	2	3	4	5	6
(1) Bahamas		0.921	0.574	0.578	0.564	0.558
(2) Rocas Atoll	0.082		0.592	0.607	0.625	0.665
(3) Ceará	0.555	0.553		0.985	0.986	0.968
(4) Rio Grande do Norte	0.547	0.545	0.016		0.995	0.963
(5) Paraíba	0.572	0.535	0.014	0.005		0.969
(6) Rio de Janeiro	0.584	0.515	0.033	0.038	0.032	

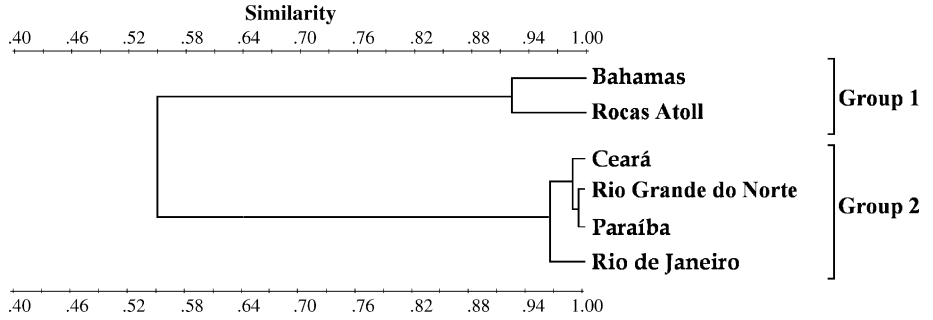

Fig. 2. UPGMA tree based on unbiased allozyme identities (Nei, 1978) of the populations analysed. The two groups, corresponding to two different species of *Bathygobius*, are indicated.

3). The nucleotide composition of cytochrome *b* sequences was 22.8% adenine, 29.4% thymine, 31.3% cytosine and 16.5% guanine. The two groups could be distinguished by 55 diagnostic nucleotide substitutions (Fig. 3). The genetic distances (Kimura 2-parameters, K2P) ranged from 0.00 (between sequences from the Rocas Atoll) to 0.27 (between sequences from the specimens Bahamas #19 and Rio de Janeiro #01) (Table 4). The topology of the tree based on mitochondrial data (Fig. 4) is congruent with that produced with allozymes (Fig. 2); on both trees the highest differences were found between the Bahamas/Rocas Atoll group and the Brazilian continental samples. Those two groups were also strongly supported by the AMOVA analyses, with 82% (allozymes) and 56% (cytb) of the total variance found between them (Table 5).

4. Discussion

The insular populations from the Bahamas and Rocas Atoll populations were genetically highly differentiated from those, supposedly conspecific populations, from the Brazilian continental coast both for the nuclear (five diagnostic allozyme *loci*) and mitochondrial (K2P distances >16%) genes. The AMOVA also assigned most of the allozyme and cytochrome *b* variation of the populations to differences between the two groups. This clearly shows that these two groups are evolving independently and represent two distinct species. Nevertheless, the meristic characters traditionally used to separate West Atlantic *Bathygobius* species in taxonomic keys fail to distinguish the two species found here.

Five diagnostic allozyme *loci* were observed between the Bahamas/Rocas Atoll and the coastal Brazilian populations. The mean genetic identity observed between the two groups (0.55; Fig. 2) is typical of congeneric comparisons (Thorpe, 1982). More significantly, it is lower than that found between the East Pacific *Bathygobius ramosus* and *Bathygobius andrei* (*I*=0.66; Gorman et al., 1976) and between *B. soporator* and *B. andrei* (*I*=0.86; Gorman et al., 1976). Those two last species are found, respectively, on the Atlantic and Pacific sides of Panama, which means that they have diverged for at least 3.5–4.5 million years, since the rise of the Isthmus of Panama (Coates et al., 1992; Knowlton and Weigt, 1998). Therefore, it is likely that the two Atlantic species found here, within *B. soporator*, have diverged well before the complete separation between the Atlantic and the Pacific oceans.

The level of nucleotide divergence found between the two groups of *Bathygobius* '*soporator*' (K2P distance=0.21) is also very high, similar or higher to that found in comparisons of gobiid (*Gymnogobius* 0.12–0.21, Harada et al., 2002) and other fish species (*Amphiprion* 0.05–0.13, Elliott et al., 1999; *Ophioblennius* 0.13–0.16, Muss et al., 2001). The Bahamas/Rocas Atoll and the continental Brazilian populations clearly belong to different species. Since the type locality of *B. soporator* is Martinique in the Caribbean (Cuvier and Valenciennes, 1837) we recommend that the binomial be maintained for the Bahamas and Rocas Atoll populations. The dispersal capability of *B. soporator* has never been verified, but this species exhibits the common gobioid pattern, having adhesive demersal eggs, nest-guarding by the male, and a larval life of 30 days (Tavolga, 1950;

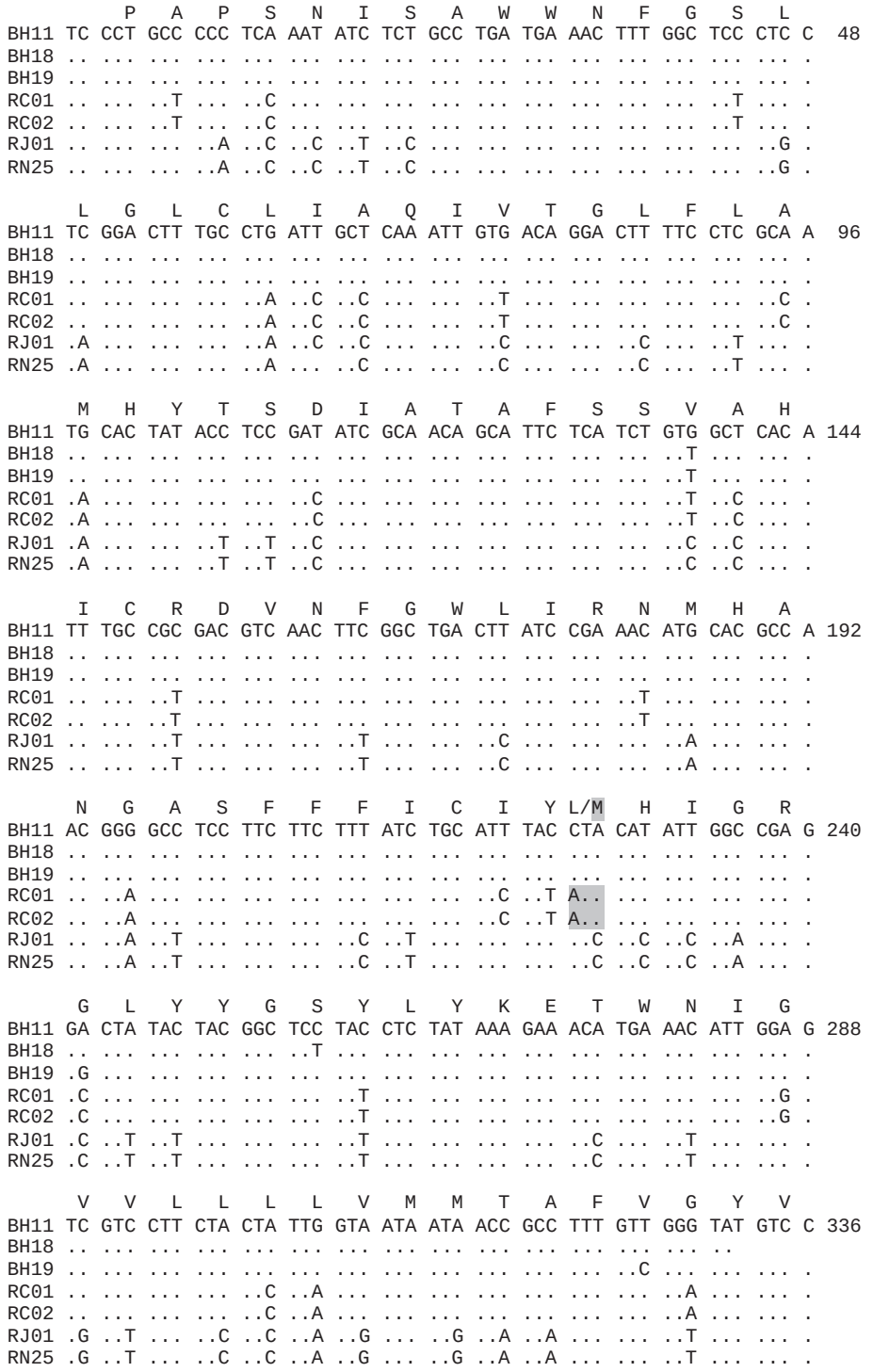

Fig. 3. Alignment of partial cytochrome *b* sequences and corresponding amino acid sequences of *Bathygobius*. Marked in grey are non-synonymous substitutions (BH=Bahamas; RC=Rocas Atoll; RJ=Rio de Janeiro; RN=Rio Grande do Norte).

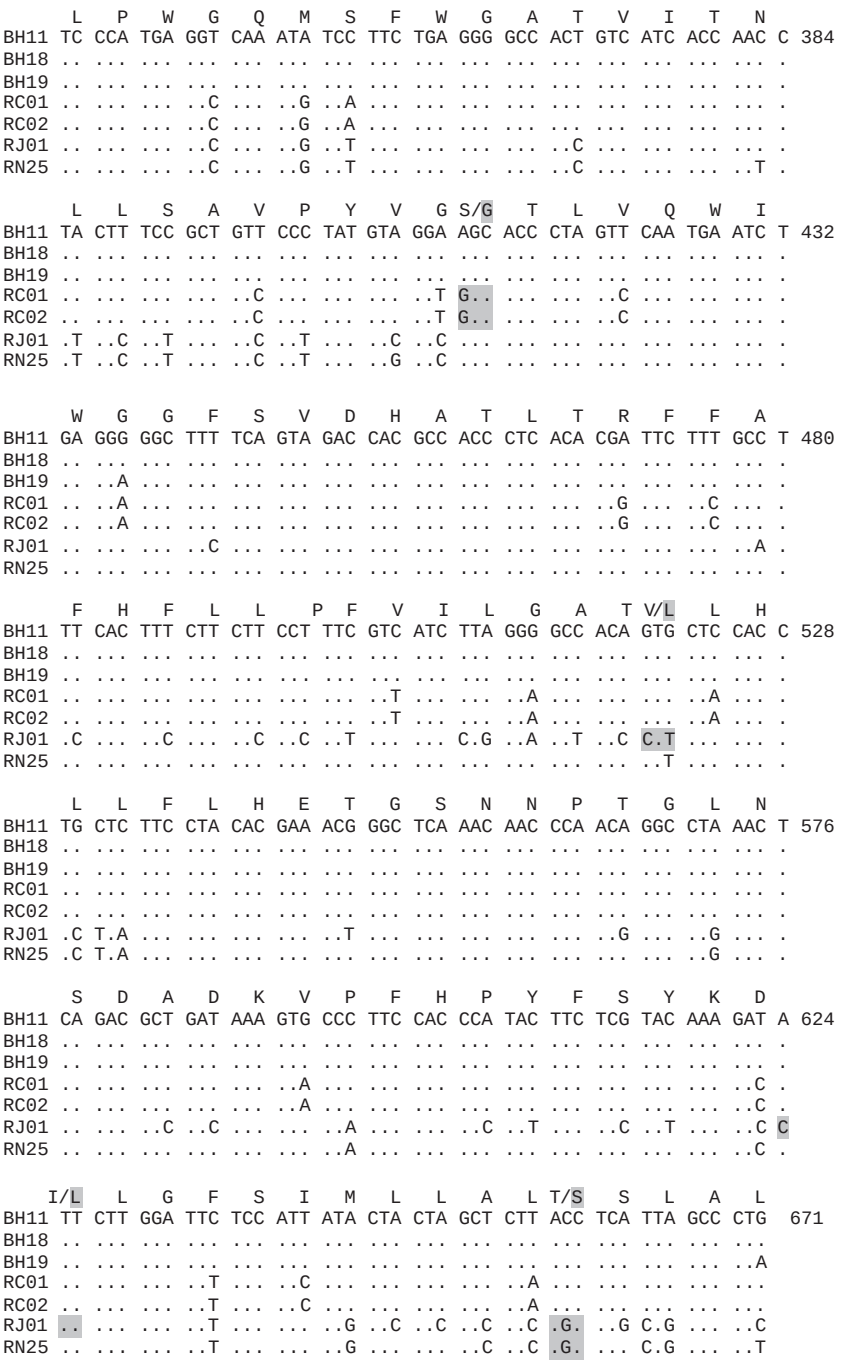

Fig. 3 (*continued*).

Peters, 1983). This indicates that this species has very poor dispersal capabilities, like other gobiid species (Shulman and Bermingham, 1995; Leis et al., 2003; Taylor and Hellberg, 2003). The other Brazilian populations of *Bathygobius* analysed either represent the occurrence of a species of *Bathygobius* hitherto

Table 4
Kimura 2-parameter distances (below diagonal) and standard errors (above diagonal) values between pairs of cytochrome *b* sequences of *Bathygobius soporator*

Samples	1	2	3	4	5	6	7
1) Bahamas 11		0.002	0.003	0.014	0.014	0.040	0.026
2) Bahamas 18	0.003		0.003	0.014	0.014	0.041	0.027
3) Bahamas 19	0.008	0.008		0.014	0.014	0.041	0.027
4) Rocas Atoll 01	0.079	0.079	0.079		0.000	0.039	0.028
5) Rocas Atoll 02	0.079	0.079	0.079	0.000		0.039	0.028
6) Rio de Janeiro 01	0.258	0.265	0.270	0.250	0.250		0.010
7) Rio Grande do Norte 25	0.163	0.168	0.171	0.171	0.171	0.050	

Gamma correction: γ=0.298.

unknown for the Atlantic (which is unlikely, given the poor dispersal capabilities of the species) or belong to a new species. Thus, we will use the name *Bathygobius* sp. for the samples morphologically similar to *B. soporator* found on the coast of mainland Brazil, until the taxonomic status of that species is clarified.

The species *B. mystacium* is morphologically very similar to *B. soporator* and one could consider the possibility that the Brazilian samples might belong to that species. However, the morphological differences used to discriminate between those two species (the number of lateral scales and the maxilla length/standard length (ML/SL) ratio; Bohlke and Chaplin, 1993) unambiguously place all of our samples within the description of *B. soporator*. All fish collected in the Caribbean and in Brazil had 37–41 lateral scales and a maxilla length/standard length (ML/SL) ratio of 12–14%, whereas the corresponding numbers for *B. mystacium* are 33–36 scales and a MS/SL ratio of 9–12% (Table 1; Bohlke and Chaplin, 1993).

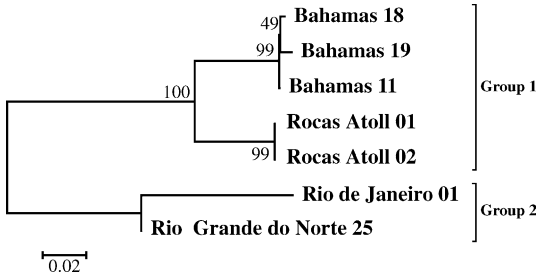

Fig. 4. Neighbor-joining tree based on Kimura 2-parameter distances for the populations analysed. The two groups, corresponding to two different species of *Bathygobius*, are indicated. Bootstrap values (1000 permutations) given on branches.

The other species of *Bathygobius* known for the Caribbean region is *B. curacao* (Bohlke and Chaplin, 1993), but the samples analysed in this study were very different from the description of that species, which has an even lower number of lateral scales (31–34). Also, *B. curacao* has 15–18 soft rays on the pectoral fins, whereas the fish analysed in this study had 20–21 soft rays (Table 1). The two other recognised Atlantic species of *Bathygobius* occur on the African coast: *B. burtoni* and *B. casamancus*. Those two species can be distinguished from *B. soporator* because of their lower maxilla length/standard length ratio (*B. soporator*=12–14%; *B. burtoni*=9–11%; *B. casamancus*= 8–9%; Miller and Smith, 1989) and number of pre dorsal scales (*B. soporator*=22–24; *B. burtoni*=13–18; *B. casamancus*= 14–21; Miller and Smith, 1989) (Table 1).

The genetic data indicate that *B. soporator* and *Bathygobius* sp. have diverged for a long time. Sequences of cytochrome *b* in fish diverge of about 2% per MY (Brown, 1985; Brown et al., 2001). This suggests that the two *Bathygobius* species studied

Table 5
Analysis of molecular variance of the island (Bahamas+Rocas Atoll) and Brazilian continental (Ceará+Rio Grande do Norte+Paraíba+Rio de Janeiro) groups, based on allozyme and cytochrome *b* data

Source of variation	Allozyme		Cytochrome *b*	
	Variance components	% of variance	Variance components	% of variance
Among groups	4.16	81.85	24.58	56.19
Among populations within groups	0.24	4.63	17.83	40.76
Within populations	0.69	13.52	1.33	3.05

here have diverged since around 11–12 MY bp. A remarkably similar value (10 MY) is observed with the allozyme data, if we consider the calibration of 1 D=18 MY obtained in the comparison Panamanian populations of *B. soporator* (from the Atlantic) and *B. andrei* (from the Pacific; Gorman et al., 1976). The major marine barrier between the Caribbean and Brazil is the outflow of Amazon–Orinoco Rivers, which also started about 11 MY bp (Hoorn, 1993). This important barrier may have influenced the formation of many fish sister species between the Caribbean and Brazil regions (e.g. Greenfield, 1988; Moura et al., 2001; Muss et al., 2001; Rocha and Rosa, 2001a; Guimarães and Bacellar, 2002; Rocha et al., 2002), including the species of *Bathygobius* analysed here.

The samples from Rocas Atoll were genetically similar to those from the Bahamas (5700 km northwest from Rocas). The Rocas Atoll is about 4.8 MY old (Gomes et al., 2001), but the similarity values for both the allozyme and cytochrome *b* data indicate that the colonization of the Rocas Atoll must have happened much more recently. The oceanic islands of Bahamas and the Rocas Atoll have more similar environmental conditions than those of continental coastal areas (Buchan, 2000; Leão and Dominguez, 2000), which are strongly influenced by river discharges. The reef fish community of Rocas Atoll has one of the most divergent trophic structure patterns in the Southwest Atlantic, with a predominance of planktivores, in contrast with roving herbivores at low latitudes and omnivores at high latitude reefs (Ferreira et al., 2004). Other sister fish species exhibit the same pattern of distribution; for example, *Halichoeres radiatus* (Labridae) occurs in the Caribbean and in the oceanic islands of St Paul's Rocks (off North Brazil), Fernando de Noronha archipelago (off Northeast Brazil) and Rocas Atoll, whereas its sister species, *Halichoeres brasiliensis*, is distributed only along the Brazilian tropical coast (Rocha and Rosa, 2001b). The same is observed with the gobies *Elacatinus randalli* (Caribbean and Fernando de Noronha) and *Elacatinus figaro* (restricted to the Brazilian coast; Sazima and Moura, 2000), and the grunts *Haemulon chrysargyreum* (from the Caribbean, Fernando de Noronha and Rocas Atoll) and *Haemulon squamipinna* (Brazilian coast) (Rocha and Rosa, 1999).

5. Conclusions

The detection of a genetically highly divergent, but cryptic species within *B. soporator* indicates that its accepted distribution should be narrowed. The discovery, here, of a limited dispersal capability in the species is congruent with what has been observed in other gobiid species (Taylor and Hellberg, 2003). It is possible, therefore, that the specimens identified as *B. soporator* on the West African coast are also misidentifications, and should have their taxonomic situation verified, both morphologically and molecularly.

The reasons for the higher similarity of fish species from the oceanic islands off the North and Northeast Brazilian coast with those from the Caribbean region, rather than with those from the geographically much closer areas of coastal Brazil is puzzling. This phenomenon could be related to the peculiar ecological conditions of those islands (absence of freshwater outfalls, low primary productivity, and a high percentage of planktivory Rocha, 2003; Ferreira et al., 2004).

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