

Early Pleistocene lineages of *Bagre bagre* (Linnaeus, 1766) (Siluriformes: Ariidae), from the Atlantic coast of South America, with insights into the demography and biogeography of the species

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Coastal and marine environments are characterized by a lack of evident physical barriers or geographic isolation, and it may be difficult to understand how divergence can arise and be sustained in marine environments. The identification of ‘soft’ barriers is a crucial step towards the understanding of gene flow in marine environments. The marine catfishes of the family Ariidae are a demersal group with restricted migratory behavior, no pelagic larval stages, and mechanisms of larval retention, representing a potentially useful model for the understanding of historical processes of allopatric speciation in the marine environment. In the present study, two lineages of the Coco sea catfish, *Bagre bagre*, were recognized from their complete segregation at both mitochondrial and morphological levels. One lineage is distributed between Venezuela and the northern coast of Brazil, including the semiarid northeast coast, while the second lineage is found on the eastern coast of Brazil, including the humid northeast coast. Based on distribution area, habitats preference, and genetic variability, inferences are made in relation to biogeography and demography of lineages in Atlantic coast of South America.

Ambientes marinhos costeiros são caracterizados pela falta de barreiras físicas evidentes e ausência de isolamento geográfico, sendo difícil compreender como diferentes linhagens podem surgir e ser mantidas nestes ambientes. A identificação de barreiras permeáveis é um passo importante para a compreensão do fluxo gênico em ambientes marinhos. Os bagres marinhos da família Ariidae são um grupo demersal, com mecanismos de retenção larval e sem comportamento migratório ou estágio larval pelágico, representando um grupo potencialmente útil para a compreensão de processos históricos de especiação alopátrica no ambiente marinho. No presente estudo, duas linhagens do bandeirado ou bagre de fita, *Bagre bagre*, são reconhecidas com base na completa segregação mitocondrial e morfológica. Uma linhagem é encontrada da Venezuela à costa norte do Brasil, incluindo a costa nordeste semi-árida, enquanto a segunda linhagem é encontrada na costa leste e sudeste do Brasil, incluindo a costa nordeste úmida. Com base na área de distribuição, habitat preferencial e a variabilidade genética, inferências são feitas em relação à biogeografia e demografia das duas linhagens na costa do Atlântico da América do Sul.

Keywords: Genetics, Morphological, Pleistocene, Sea level variations, Species evolution.

Introduction

Understanding the processes that determine the differentiation of closely related species, not delimited by obvious physical barriers, represents one of the greatest challenges in evolutionary biology (Pampoulie *et al.*, 2004). Coastal and marine environments are characterized by a lack of evident physical barriers or geographic isolation (Bellwood & Wainwright, 2002; Rocha *et al.*, 2002; Luiz *et al.*, 2012), making it difficult to understand how closely related species can arise and be sustained in marine environments (Hauser

& Carvalho, 2008). The identification of ‘soft’ barriers, such as the configuration of ocean currents, temperature gradients, and geomorphological features of the coastline, together with the biological characteristics of each species, is a crucial step towards the understanding the processes responsible for the differentiation and maintenance of close species in marine environment.

Demersal species with restricted migratory behavior, no pelagic larval stages and mechanisms of larval retention can represent an excellent model for the understanding of the process of allopatric speciation in the marine environment

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(Stepien *et al.*, 2001; Betancur-R *et al.*, 2010). The sea catfishes of the family Ariidae inhabit shallow coastal waters, migrating to estuaries and/or coastal floodplains to spawn (Rimmer, 1985; Araújo, 1988), when the males retain the fertilized eggs in the oral cavity for up to three months, when they do not feed, after which the young remain in estuarine habitats until they mature (Rimmer, 1985; Araújo, 1988).

The Coco sea catfish, *Bagre bagre* (Linnaeus, 1766) is widely acknowledged as a valid species found on the Atlantic coast of South America, from Colombia to southeastern Brazil (Marceniuk & Menezes, 2007; Marceniuk *et al.*, 2015). The Coco sea catfish is known to practice oral incubation and lack pelagic larval stages (Le Bail *et al.*, 2000), but is rare from coastal lagoons and estuaries (Pinheiro *et al.*, 2006; Dantas *et al.*, 2010; Mendes & Barthem, 2010; Le Bail *et al.*, 2012; Barbanti *et al.*, 2013). In contrast with other ariids, which are typically endemic to relatively restricted geographical areas (Betancur-R. & Armbruster, 2009), species of the genus *Bagre* are very amply distributed (Marceniuk & Menezes, 2007), considered to be a characteristic of invasive species (Lodge, 1993). In some parts of Brazil, such as the state of Pará, this species is targeted intensively by industrial fisheries, while in other regions, there are only limited artisanal catches (Marceniuk *et al.*, 2015).

In the present study, two early Pleistocene lineages of the Coco sea catfish are recognized based on molecular and morphological evidence. Considering the area of distribution, genetic variability, and habitat preferences, inferences are made in relation to biogeography, demography, and conservation of these two lineages from the Atlantic coast of South America.

Material and Methods

Study area and distribution data. Considering the role of climatological and oceanographic factors in the determination of patterns of biodiversity (Amaral & Jablonski, 2005), five distinct regions were recognized along the Brazilian coast for the purposes of analysis in the present study: (i) the northern coast, between the mouth of the Oiapoque River (4° N) in the state of Amapá and the mouth of the Parnaíba River (3° S) in Maranhão, (ii) the northeastern coast, between the Parnaíba and Todos os Santos Bay (13° S) in Bahia, (iii) the eastern coast, between Todos os Santos Bay and the Cape of São Tomé (22° S) in the state of Rio de Janeiro, (iv) the southeastern coast, between São Tomé and the Cape of Santa Marta (28° S) in Santa Catarina, and (v) the southern coast, between Santa Marta and the Chuí (34° S) in Rio Grande do Sul (*sensu* Ekau & Knoppers, 1999, Fig. 1). The region between the mouth of the Orinoco River (8° N) in Venezuela, and French Guiana (4° N), dominated by the plume of the Amazon and Orinoco rivers, is considered to be contiguous with the northern coast of Brazil. The coast of northeastern Brazil can be divided into two distinct sectors, with semi-arid conditions on the north-facing sector, between Ceará (3° S) to Rio Grande do

Norte (6° S) States, where mean annual precipitation is less than 800 mm, and local conditions are influenced by the Northern Brazilian Current, and more humid conditions on the east-facing sector, between Paraíba (6° S) and Bahia (13° S) States, which is influenced by the Brazilian current, and has a humid tropical climate, with annual precipitation of over 1,000 mm (Maia *et al.*, 2006) (Fig. 1).

Data on the distribution of the Ariidae on the Atlantic coast of South America were obtained from the catalogs of the zoological collections available from the Neodat (<http://www.mnrj.ufrj.br/search.htm>), SpeciesLink (<http://www.splink.org.br/>), and FishNet (<http://www.fishnet2.net>) projects, and the ichthyological collection at the Museu Paraense Emílio Goeldi (MPEG).

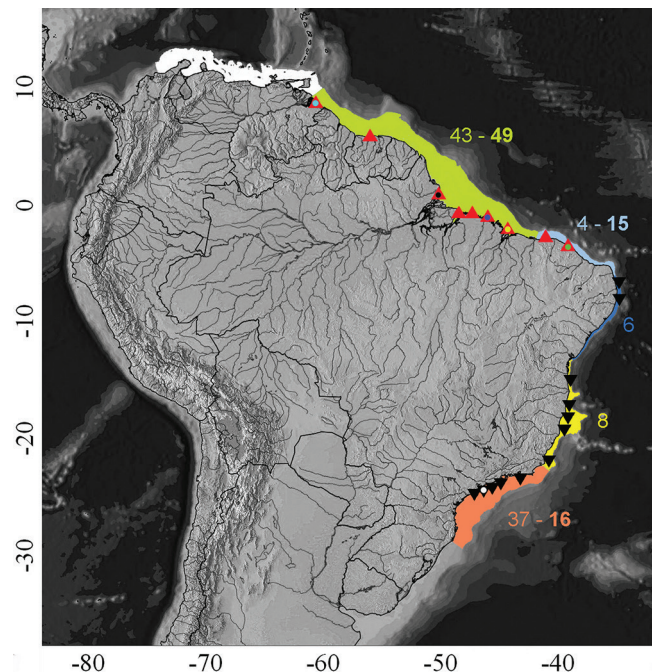


Fig. 1. Geographic distribution of the two *Bagre bagre* lineages found in South America [red triangle = northern Brazilian coast, including region dominated by the plume of the Amazon and Orinoco rivers and semi-arid sector of Brazilian northeast coast (light blue = mouth of the Orinoco River; black circle = Macapá, AP; dark blue circle = Bragança, PA; yellow = São Luis, MA; green = Fortaleza, CE), and inverted black triangle = eastern and southern Brazilian coast, including the humid sector of the northeast coast (white circle = Santos, SP)] showing the number of specimens analyzed in each region (not bold = morphological analysis, and bold = molecular analysis). Green = northern Brazilian coast influenced by the Amazon–Orinoco plume, Light blue = semi-arid northeastern coast of Brazil; Dark blue = humid northeastern coast of Brazil, Yellow = east coast, and Orange = southeast coast).

DNA sequence data. Molecular analyses were based on *Bagre bagre* tissue samples obtained from 80 specimens collected in Brazil on the northern coast, the semi-arid northeastern

coast, and the southeastern coast (Appendix 1) (Fig. 1). Two databases were established, one used for the population-level analyses, which contained only these specimens, and the other containing the known *B. bagre* haplotypes as well as two specimens of *Bagre marinus* (Mitchill, 1815) from the semi-arid northeastern coast of Brazil, in addition to DNA sequences of specimens of the families Ancharidae and Ariidae obtained from GenBank (Appendix 1). Voucher specimens were deposited in the zoological collections of the MPEG and the Laboratório de Biologia e Genética de Peixes (LBP) do Departamento de Morfologia, Instituto de Biociências da UNESP de Botucatu (Appendix 2).

Total genomic DNA was obtained using a phenol/chloroform modified from Sambrook & Russell (2001). Sequences of the Cytochrome *b* and ATPase Alpha 8/6 genes were amplified using the Polymerase Chain Reaction (PCR). The sequences of the primers and the conditions of amplification of each gene used in this study were described by Betancur-R *et al.* (2007). The PCR products were purified using an ExoSAP-IT kit (Amersham Pharmacia Biotech) and sequenced using Big Dye 3.1 in an ABI 3500 automatic sequencer, following the maker's instructions (Applied Biosystems, Foster, CA, USA).

Phylogenetic inference. The DNA sequences were aligned using the ClustalW multiple alignment tool (Thompson *et al.*, 1997) in BioEdit v. 5.0.6 (Hall, 1999). Following the automatic alignment, the sequences were inspected visually for the correction of possible incongruities.

Bayesian Inference (BI) and a Maximum Likelihood (ML) analyses were run in Mr. Bayes v. 3.1.2 (Ronquist & Huelsenbeck, 2003) and PhyML v. 3.0 (Guidon *et al.*, 2010), respectively. As both markers are part of the mitochondrial genome (single linkage) part of the database was concatenated (Cytb+ATPase 8/6-1537 bps) in the BioEdit program. The database was analyzed with a single (non-partitioned) substitution model, and partitioned by gene and codon position (substitution models were selected for each of the three codon positions of each gene). The most adequate substitution models were selected by a second order Akaike Information Criterion using a hierarchical likelihood test, as well as the Bayesian Information Criterion, which was estimated in jModeltest 2 (Darriba *et al.*, 2012). In the ML analysis, reliability of the branches was tested using 1000 nonparametric bootstrap replicates (Felsenstein, 1985). For BI, the database was analyzed as a single model of substitution (unpartitioned) and partitioned by gene and codon position (separate substitution models were selected separately for each partition of the genes and the concatenated dataset). Analyses in Mr Bayes 3.1.2 were based on MCMC sampling in four simultaneous runs, each consisting of four chains (one cold and three heated) with a total run of 10 million generations. Bayesian probabilities were selected based on a 60% consensus, with random initial trees, which were sampled at every 100 generations. Conservatively, 25% of the first trees sampled in each MCMC run were discarded

as burn-in. The log-likelihood scores were then plotted in Tracer v. 1.4 (Rambaut & Drummond, 2009) to confirm the validity of the burn-in period initially used in Mr. Bayes. The post burn-in samples were used to construct a strict consensus tree.

Estimates of divergence times. Divergence times were estimated in BEAST v. 1.7.4 (Drummond & Rambaut, 2009). Initially, the BEAST file was built in BEAUti program (Drummond *et al.*, 2007). The substitution model utilized was HKY (selected for AIC), with base frequencies estimated empirically, using the database partitioned by codon. All parameters were unlinked and mean substitution rate was unfixed. For the tree prior parameters, two simulations were performed. Initially, the tree prior Speciation: Yule Process was used. This simplest branching model assumes that, at any given point in time, every living lineage can speciate at the same rate, λ . Because the speciation rate is constant through time, there is an exponential waiting time between speciation events (Yule, 1924; Aldous, 2001), being more appropriated for species-level phylogenies (Drummond *et al.*, 2007). This model does not allow for extinctions. We also performed simulations utilizing the tree prior Birth-Death process, which is an extension of the Yule process. The Birth-Death model assumes that at any point in time every lineage an undergo speciation at rate λ or go extinct at rate μ . These two simulations were run using strict clock and the Calibration nodes were constrained using lognormal-distributions priors.

The calibration of the nodes was chosen based on the paper of Betancur-R. & Armbruster (2009). These are the only calibration points available for marine catfish in the literature, so we implement these calibration points to estimate our target data (the split between the two lineages of *B. bagre* found in our study). Those calibration points were: (i) 73 million years for the separation of *Gogo arcuatus* from the Ariidae (*Gogo arcuatus* + all ariid specimens), (ii) 23 million years for the oldest fossil of *Cathrops*, which separates this genus from the other ariids included in the study (*Cathrops* + *Galeichthys/Bagre*), and (iii) 3 million years for the uprise of the Isthmus of Panama, the vicariant event that separated the modern *Cathrops* species *C. mapale* and *C. fuertii* from *C. dasycephalus* (*C. mapale/C. fuertii* + *C. dasycephalus*). For this calibration point, the date of the closure itself has recently been debated based on new geological and molecular data, arguing for an old closure time (Bacon *et al.*, 2015), rather than the classically accepted 3.1-3.5 Mya (Knowlton *et al.*, 1993; Collins, 1996; Lessios, 2008). Taking those debates under account, we also using 5 mya as the vicariant event of modern *Cathrops* species. The MCMC method was used to infer divergence times between the northern and southern populations, run in BEAST 1.7.4 (Drummond & Rambaut, 2007). This was based on two independent MCMC runs of 160 million interactions, with samples being taken every 1,000 generations. The MCMC log files were combined in Tracer to summarize posterior

divergences times with 95% highest posterior density limits. For our study, only runs where the ESS values were greater than 200 were reached for all marginal parameters after discarding 10% of the first trees as burn-in.

Diversity indices and demographic history. Indices of haplotype (H) and nucleotide diversity (Nei, 1987), as well as Tajima's (1989) neutrality test (D) and Fu's (1997) F_s were all estimated in DnaSP, v. 5.1 (Librado & Rozas, 2009). The fixation indices (Φ_{st} ; Weir and Hill, 2002) and hierarchical analysis of molecular variance (AMOVA; Excoffier *et al.*, 1992) based on 1000 permutations using the Kimura 2P substitution model (Kimura, 1980) were obtained in Arlequin v. 3.11 (Excoffier *et al.*, 2005), as were the pairwise differences between localities. The genealogical relationships among the haplotypes were defined by a haplotype network, constructed in the Haploviewer program (Salzburger *et al.*, 2011) using the Maximum Likelihood algorithm obtained in JmodelTest 2 (Darriba *et al.*, 2012).

The demographic history of *Bagre bagre* in the western South Atlantic was reconstructed in a Bayesian framework using the Bayesian Skyline Plot (BSP), run in BEAST v. 1.7.4 (Drummond & Rambaut, 2007). These analyses use the Monte Carlo Markov Chain (MCMC) method, based on nucleotide sequence data to estimate posterior distributions of effective population size (N_e) over time. The evolutionary model used to simulate the BSP curves was selected in jModelTest 2 (Darriba *et al.*, 2012). The best evolutionary model selected was that of Hasegawa-Kishino-Yano with invariable sites (HKY+I) (-lnL= 1072.13, BIC= 4201.87) (HKY+I). Two independent runs of 100,000,000 MCMC interactions were conducted, with samples being taken every 1000 generations. The first 10 million samples were discarded as burn-in. The program LogCombiner v. 1.7.4 (Rambaut & Drummond, 2009) was used to combine the independent runs to produce graphic representations of the BSP.

Morphological analyses. The analysis of morphological variation in the *Bagre bagre* group was based on both morphometric and meristic characters. Forty-seven measurements were taken from 98 specimens (Fig. 1, Appendix 2), following the diagnostic approach of Marceniuk (2007). Meristic counts were made of the anal fin rays and the rakers of the first and second gill arches. Quantitative measurements were taken using either a ruler (to the nearest millimeter) or dial callipers (precision of 0.1 mm). The data were analyzed using XLStat (Microsoft Excel), following the log-transformation of the co-variance matrix. Variables that were constant, fluctuated randomly or varied according to body size or sex, were excluded from the PCA.

The specimens examined in the morphological study were obtained from the zoological collections of LBP, MPEG, Museu de Zoologia da Universidade de São Paulo (MZUSP), and National Museum of Natural History at the Smithsonian Institution, Washington DC (USNM) (Appendix 2).

Ethics Statement. The species analyzed in the present study are not endangered or protected in the regions from which the specimens were obtained. All the specimens were caught by local fishermen, and were handled in strict accordance with practices defined by the relevant local animal welfare body.

Results

Phylogenetic analyses and divergence times. A total of 769 base pairs (bps) were sequenced from the Cytb gene, and 758 bps from the ATPase 8/6 gene, resulting in a concatenated dataset of 1527 bps. The TIM2+G evolutionary model was selected for both the maximum likelihood and Bayesian inference trees, and their topologies were highly similar, to the extent that only the Bayesian tree is presented here (Fig. 2). The topologies provide evidence of the existence of two *B. bagre* lineages, one from the northern Brazilian coast (including the semi-arid sector of the northeast coast, following morphological data, see below) and one from the eastern and southern Brazilian coast (including the humid sector of the northeast coast, following morphological data, see below) (Figs. 1-2). The two clades are supported by ML (100%) and BI (1.0) values. The Bayesian analysis of the most recent common ancestor (Fig. 3) indicated that the separation of the two *B. bagre* lineages occurred approximately 1.7 mya (range: 1.11-2.44 mya).

Haplotype diversity and demography. A high degree of haplotype diversity was found in the population from the Brazilian northern and northeast semi-arid coast, whereas genetic diversity was very low in the stock from Brazilian southeastern coast (Fig. 4; Table 1). As the observed pattern of haplotype diversity may have been influenced by the difference in sample size between the two lineages, given that the northern lineage was represented by 64 specimens, and the southeastern lineage, by only 16 specimens, comparisons of the diversity of sites with the same sample size (Maranhão and Ceará States) were run, although they produced results that were highly similar to those of the general analysis (Table 1). Estimated haplotype diversity for the 16 specimens from southeastern lineage (São Paulo State) was 0.35 (0.135), while the values recorded for Maranhão (16 specimens) and Ceará (15 specimens) were more than twice this, at 0.85 (0.077) and 0.76 (0.009), respectively. It is important to note that the difference in the sample size for the populations of eastern and southern Brazil was not due to sampling deficiencies, but rather the naturally reduced abundance of the species in this region, a well-documented phenomenon (Dantas *et al.*, 2010; Marceniuk *et al.*, 2015).

The tests of equilibrium of mutation/drift (Tajima's D and Fu's F_s) returned negative, but not significant values, when the population of each locality was considered separately. However, given the initial results, which indicated that the northern Brazilian lineage represents a distinct species from that of the eastern and southern coast of Brazil, considering that the F_s were negative and significant considering all the localities from the northern and northeastern coast together (Table 1).

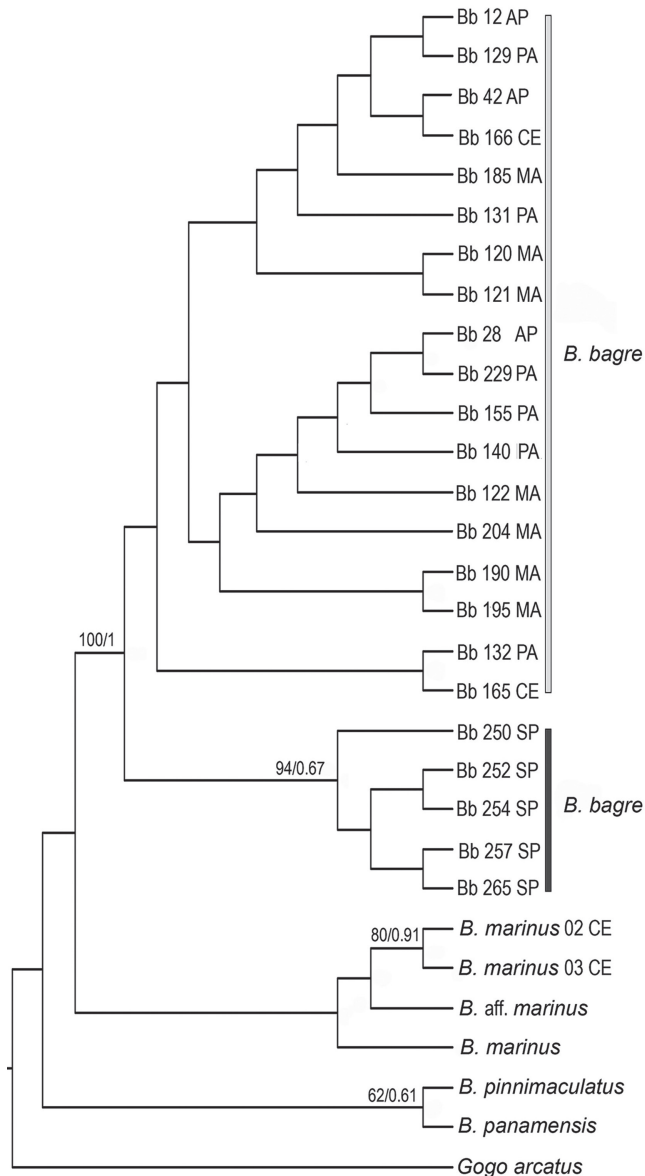


Fig. 2. Phylogenetic tree of the Bayesian Inference of the *B. bagre* derived from the concatenated database of the Cytb 1 and ATPase 8/6 genes. Only unique haplotype are included here. The first value on each branch corresponds to the ML support value and the second one to the BI. Only support values and posterior probabilities above 60% are shown. Grey = northern Brazilian coast lineage, including the semi-arid sector of the northeast coast; Black = southern Brazilian coast.

The greatly reduced genetic variability found in the São Paulo lineage meant that the ESS values obtained from our BEAST simulations were not statistically reliable enough to reconstruct the BSP curves for this lineage. Given this, the BSP curves are presented here only for the northern Brazilian lineages. The curves of the BSP also indicate population expansion in both genes. The population of the northern Brazilian lineage underwent an expansion approximately 10,000 years

ago, during which its effective size (N_e) increased from $\sim 374861-11$ (29.882-12 to 1660690-10) to $\sim 464295-10$ (33262-12 to 2144211-9) at 7500 years ago, to $\sim 925823-10$ (79277-11 to 4356937-10) around 1500 years ago (Fig. 5A). This expansion was more discreet for the ATPase 8/6 data, but the period coincided with that of the Cytb gene (Fig. 5B). Due to the reduced genetic variability of the southeastern population, it was not possible to plot the BSP curve with an adequate ESS for analysis.

The F_{st} values and the results of the AMOVA also confirmed the differentiation of the Brazilian northern and northeast semi-arid coast lineage from the southeast coast lineage. The F_{st} values were not significant for all the pairs of northern coast populations, although when these populations were compared with that from the southeast coast, the values varied from 0.641 to 0.771 (Table 2). The results of the AMOVA also indicated that most of the variation (59.91%) is found between populations (Table 3). The haplotype network generated with the not connected genes indicates that haplotypes were shared by northern and northeast semi-arid coast lineage, but none of these populations shared any of the haplotypes found in the specimens from the southeastern coast of Brazil (Fig. 4).

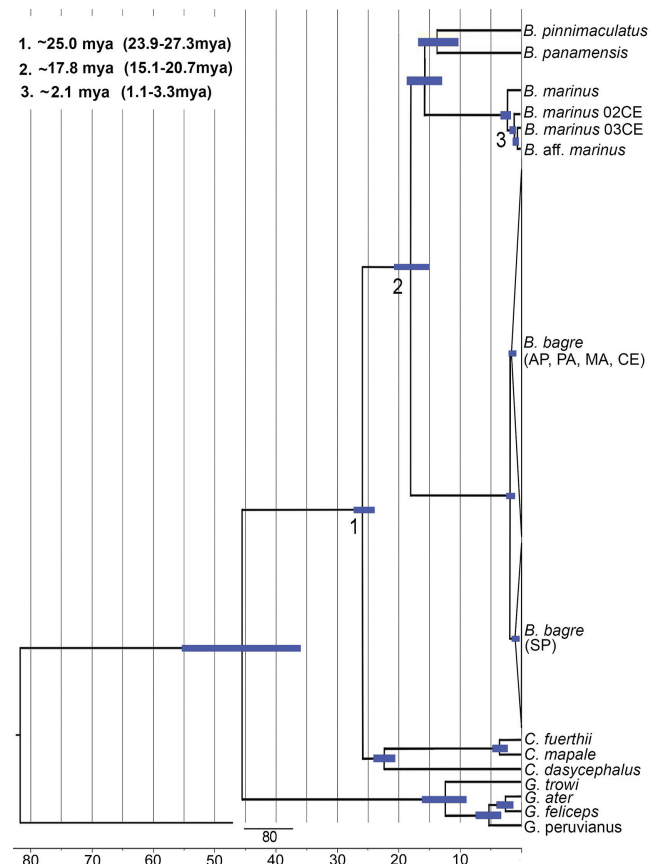


Fig. 3. Bayesian strict clock chronogram based on the 1535 bps of the concatenated genes (Cytb and ATPase 8/6). The calibration points and evolutionary rates were based on Betancur-R. & Armbruster (2009).

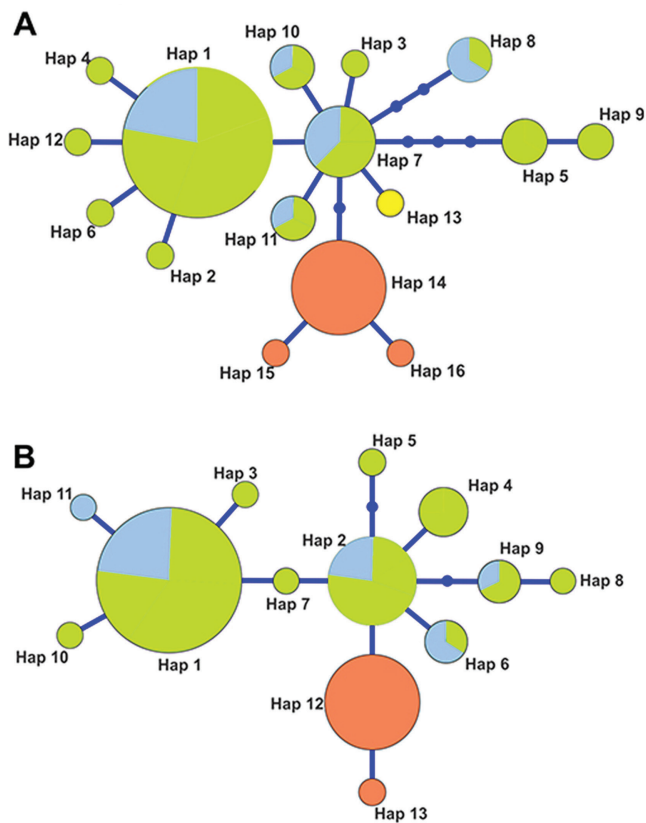


Fig. 4. Genealogies of the haplotypes of (A) the mitochondrial *Cytb* gene, based on the TIM2+I+G evolutionary model, and (B) the mitochondrial ATPase 8/6 gene, based on the HKY+G model. Green = northern Brazilian coast influenced by the Amazon–Orinoco plume, Light blue = semi-arid northeastern coast, Orange = southeastern coast.

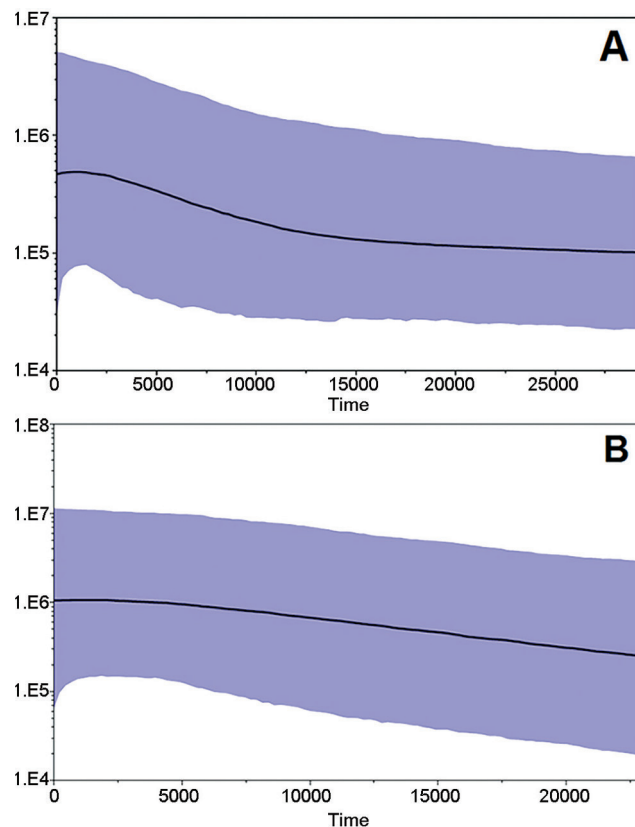


Fig. 5. Bayesian Skyline Plot for the northern lineage of *Bagre bagre*. The curves A and B correspond to the *Cytb* and ATPase 8/6 genes respectively. The y-axis indicates effective population size (N_e) vs. generation time, while the x-axis indicates the mean time in thousands of years before present.

Table 1. Diversity indices derived from the sequences of the concatenated *Cytb* and ATPase 8/6 genes analyzed in the present study from different Brazilian *Bagre bagre* populations. Abbreviations: A=Geographic areas, B=Brazilian political states, N=number of individuals; Nc=northern Brazilian coast; PS=polymorphic sites; H =haplotype diversity; π =nucleotide diversity, sa=semi-arid northeastern coast of Brazil; Sc=southeast Brazilian coast. Standard deviations are show in parentheses. *Significance value ($P < 0.05$).

A	B	N	PS	(H)	(π)	Tajima's D	Fu's F_s
	Amapá (4° N- 0°)	13	12	0.73 (0.133)	0.001 (0.000)	-1.646	-1.859
Nc	Pará (0°-1° S)	20	20	0.76 (0.103)	0.002 (0.000)	-1.502	-3.061
	Maranhão (1°- 3° S)	16	13	0.85 (0.077)	0.002 (0.000)	-0.851	-2.443
sa	Ceará (3°-5° S)	15	11	0.76 (0.009)	0.001 (0.000)	-0.772	-0.018
Nc + sa		64	27	0.77 (0.054)	0.001 (0.000)	-1.628	-14.239*
Sc	São Paulo (23°-25° S)	16	3	0.35 (0.135)	0.000 (0.000)	-1.696	-2.449
N + sa + S		80	21	0.83 (0.036)	0.002 (0.000)	-1.461	-15.765*

Table 2. Genetic divergence (F_{st}) between the species groups identified through the analysis of the *Cytb*+ATPase 8/6 genes. In the upper diagonal, the P values for F_{st} . Standard deviations are in parentheses.

Population	Amapá	Pará	Maranhão	Ceará	São Paulo
Amapá	-	0.750 (0.004)	0.224 (0.004)	0.190 (0.004)	0.000 (0.000)
Pará	-0.024	-	0.665 (0.004)	0.679 (0.005)	0.000 (0.000)
Maranhão	0.027	-0.018	-	0.446 (0.004)	0.000 (0.000)
Ceará	0.021	0.018	0.001	-	0.000 (0.000)
São Paulo	0.771	0.641	0.666	0.708	-

Table 3. Results of the Analysis of Molecular Variance (AMOVA) for the Cytb+ATPase 8/6 genes in populations of *Bagre bagre* analyzed in the present study.

Source of the variation	% of the variation
Among populations	59.91
Among populations within groups	0.21
Within populations	39.88

Morphological evidence. The Principal Components Analysis (PCA) of nine morphometric and one meristic variables was used to characterize the variation in the morphology of *B. bagre* within its geographic distribution

(Marceniuk & Menezes, 2007). The variables including in the analysis were the number of anal fin rays (AR), anal fin base length (ABL), maxillary barbel length (MaBL), mental barbel length (MeBL), mouth width (MW), body width (BW), nuchal-plate width (NPW), length of the prolongation of the pectoral fin (PP), distance from snout to dorsal fin (SDr), and distance from snout to anal fin (SAn). The PCA revealed two principal clusters, one encompassing specimens from the northern and semi-arid northeastern coast of Brazil, and the other, the populations from the humid northeast, eastern, and southeastern coasts (Fig. 6). Axis 1 explained 37.10% of total variation, and together, factors 1 and 2 explained together 50.62% of the variation (Fig. 6).

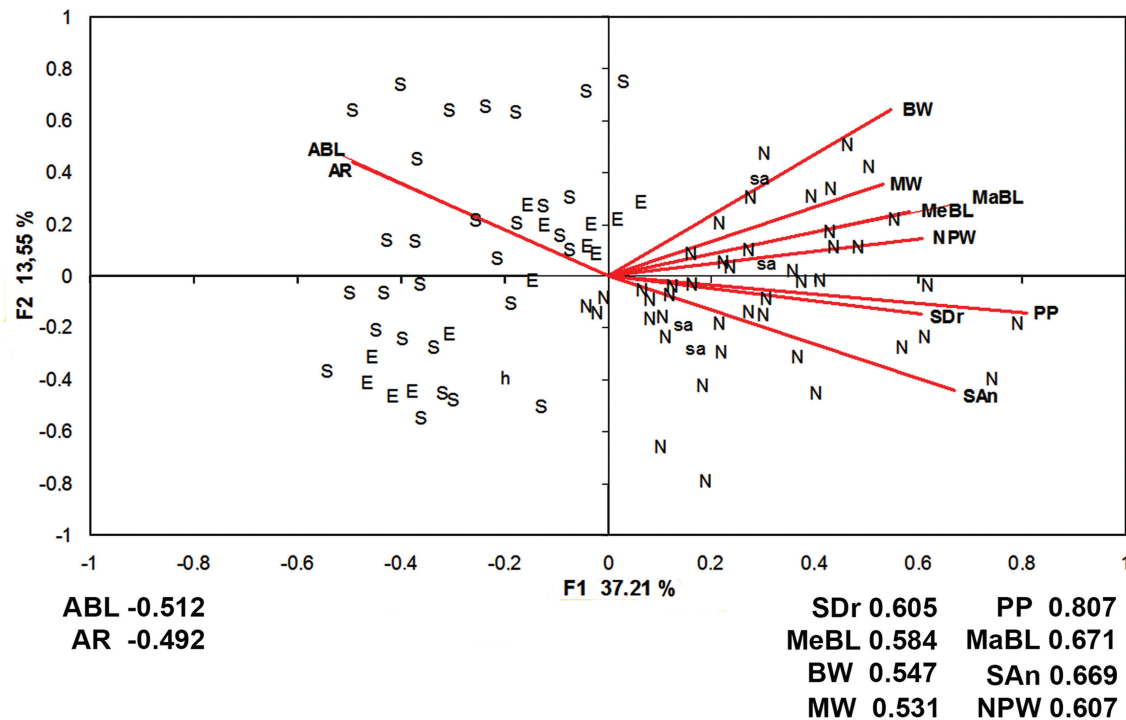


Fig. 6. Scatterplots of the principal components PC1 and PC2, obtained from the analysis of one meristic and nine morphometric variables in *Bagre bagre*, with factor loadings for the first principal components. Abbreviations: E, east Brazilian coast; h, humid northeastern coast of Brazil; N, northern Brazilian coast; sa, semi-arid northeastern coast of Brazil; S, southeast Brazilian coast.

Discussion

The molecular analysis indicated that the separation of the two *B. bagre* lineages occurred approximately 1.7 mya (range: 1.11-2.44 mya), in the early Pleistocene. The Pleistocene (2.6 mya - 11.7 ka) was a period of extreme oscillations in climate (Pillans *et al.*, 1998), with major fluctuations in global sea levels (Lambeck & Chappell, 2001; Miller *et al.*, 2005), and intense speciation in many biotas (Hewitt, 1996). The marine fauna of the shallow coastal waters and species dependent on estuaries were affected by the sea level variations, when estuarine habitats would have virtually disappeared and coastal habitats would be restricted to small enclaves, with many populations being eliminated (Grant & Bowen, 2006).

Especially in temperate southwestern Atlantic variations in sea level has been recognized as the principal factor determining habitat modifications, with coastal species being fragmented on the continental shelf of South America (Sprechmann, 1978), with marked effects on the historical demography of estuarine and coastal marine fish species (Beheregaray *et al.*, 2002; García *et al.*, 2008, 2011), resulting in the successive expansion and retraction of populations, associated with fluctuations in climate (Anderson, 2007; García *et al.*, 2008; Pereira *et al.*, 2009; Pereyra *et al.*, 2010; Fernández Iriarte *et al.*, 2011). However, comparative analyses of the southwestern Atlantic cannot provide an evaluation of the effects of Quaternary changes in sea level on the northern and northeastern Brazilian coast.

While few comparative studies are available for the northern and northeastern Brazilian coasts, Santos *et al.* (2006) also recognized two distinct lineages of *Macrodon ancylodon*, which separated in the early Pleistocene. One lineage [= *Macrodon ancylodon* see Carvalho-Filho *et al.* (2010)] occurs between Venezuela (11 °N) and the Brazilian state of Bahia (18° S), and the second lineage [= *Macrodon atricauda* (Günther, 1880) see Carvalho-Filho *et al.* (2010)] is found between the Brazilian state of Espírito Santo (18° S) and Argentina (43° S). Different patterns of replacement of the tropical clades by subtropical ones in *Bagre bagre* and *Macrodon ancylodon* may reflect differences in their adaptive responses to climate change (primarily temperature, salinity, marine currents, and fluctuations in coastal habitats) (Fernández-Iriarte *et al.*, 2011). It is important to note that, like *B. bagre* (Pinheiro *et al.*, 2006; Garcia Júnior *et al.*, 2010), *Macrodon ancylodon* is relatively rare on the northeastern coast of Brazil, in comparison with the northern and southern regions (IBAMA, 2007, 2008).

Considering allopatric speciation as the predominant geographic mode of species formation, it is reasonable to assume that vicariant differentiation may have arisen from the features of the coast of the Brazilian northeast, in particular the extremely narrow continental shelf (Fig. 1, Muehe & Garcez, 2005), which may have an enhanced effect during regression events, when it would have acted as a physical barrier between the northern and southern-southeastern coasts. This is corroborated by receding sea levels during the Pleistocene, that exposed as much as 92% of the continental shelf that is inhabitable in the present day (Ludt & Rocha, 2015). As the data offer no direct or conclusive evidence of the nature of the process lineage isolation, all potential scenarios discussed are hypothetical.

Glacial cycles and demography. The reduced haplotype diversity found in the southern lineage provides evidence of recent bottlenecks, possibly related to reductions in population size during the Holocene regression events (Rabassa *et al.*, 2005; Ruzzante *et al.*, 2008). This conclusion is supported by the significantly higher indices of nucleotide diversity found in the northern lineage (populations from the northern and semi-arid northeastern coasts of Brazil) in comparison with the southeastern lineage, *i.e.*, the humid northeastern, eastern, and southeastern coasts of Brazil (Fig. 4, Table 1). However, the analyses used to test this hypothesis lead to distinct conclusions with regard to the influence of the glacial cycles of the Holocene on the two lineages.

The results of the BSP analysis (Fig. 5) indicate a population expansion in the northern lineage around 10,000 years ago, which supports the hypothesis that the last glacial maximum played an important role in the demography of this lineage. In the Brazilian Amazon basin, two Pleistocene glacial cycles are recognized, denominated the Tucunanduba and Uatumã glaciations

(Riker *et al.*, 2015). During the Tucunanduba “Glaciation” (41,850±6380 years ago), the climate was very dry, and sea levels were 100-120 m below that of the present day, with the formation of a thick ice cap on the Guiana Shield. Following a new interglacial cycle, characterized by the deposition of the Belterra Formation, the climate once again dried and sea levels fell, initiating the Uatumã Glaciation, which followed by an interglacial period in the Holocene, which persists to the present day (Riker *et al.*, 2015). In the Brazilian state of Pará, sea levels may never have higher at any time during the Holocene than at the present time (Angulo *et al.*, 2006).

The low genetic diversity of the southeastern lineage indicates that the influence of the last glacial cycle was more pronounced in this region, due primarily to the significant loss of habitat, such as the areas of mangrove, which are limited by the 20°C isotherm temperature, leading to the abrupt reduction or disappearance of these forests (Maia *et al.*, 2006). At around 18,000 years ago, sea level was approximately 130 m below its present level, which was followed by three intermediate periods of stabilization, at 11,000 years B.P. (sea level -60 m to -70 m in comparison with the present day), 9000 years B.P. (-32 m to -45 m) and 8000 years B.P. (-20 m to -25 m) (Mahiques *et al.*, 2010). The present-day sea level may only have been reached around 7100 years ago, with much less subsequent variation (Angulo *et al.*, 2006), with the principal event occurring some 5100 years ago, when sea levels increased 4.8±0.5 m in comparison with the present day.

One other factor that needs to be taken into account is the intensive fishing on the southern and southeastern coasts of Brazil which, together with the intrinsic characteristics of the species, such as its late reproductive maturation, high longevity, low fecundity, and long period of parental care (Marceniuk *et al.*, 2015), may contribute to the reduced genetic diversity of the lineage from southeastern Brazil. The Coco sea catfish is caught only occasionally in artisanal gillnets in Santa Cruz, Espírito Santo State (18°-21° S) (Freitas Netto *et al.*, 2002) and as by-catch in shrimp trawls on the São Paulo coast (22°-25° S) (Lopes *et al.*, 2002), reflecting its reduced abundance. On the other hand, data from the Chico Mendes Institute for the Conservation of Biodiversity of the Brazilian government, indicate that the White sea catfish, *Genidens barbatus*, the principal target of the industrial fisheries of southeastern Brazil, is endangered with extinction (IUCN, 2015), based on an estimated population decline of at least 70% (<http://www.icmbio.gov.br/portal/biodiversidade/fauna-brasileira/lista-de-especies/6262-especie-6262.html>). Considering the lack of discrimination or selectivity in the exploitation of demersal resources by the region's fisheries (industrial or artisanal), it is reasonable to conclude that *Genidens barbatus* and *Bagre bagre* suffer the same pressure from fisheries on the southeastern coast of Brazil, given the similarities in the lifestyle and geographic distribution of the two species.

Habitat preferences and geographic distribution. The distribution of *Bagre marinus* and both *B. bagre* lineages indicates a disjunct spatial distribution on the Atlantic coast of South America (Fig. 7). While *Bagre bagre* is extremely common in the region influenced by the Amazon Plume (Le Bail *et al.*, 2000; Artigas *et al.*, 2003), *B. marinus* is rare or absent from this area (Pinheiro *et al.*, 2006; Garcia Júnior *et al.*, 2010) (Fig. 7). This spatially disjunct distribution may be related to preferences for distinct salinity levels, mainly determined by the freshwater discharge of local rivers (Fig. 7; Table 4).

The northern coast of Brazil is dominated by many major rivers, with a total input of approximately 1.8 x

10⁵ m³/s of freshwater (Curtin & Legeckis, 1986; Agência Nacional de Águas (ANA), 2005), and salinity may fall to as low as 32 psu during the period of peak discharge of the Amazon River, which may reach 2.4 x 10⁵ m³/s (Lentz, 1995; Grodsky *et al.*, 2014) (Table 4). This water, together with the discharge of the Orinoco River, which peaks at 7 x 10⁴ m³/s (Hu *et al.*, 2004), forms the Atlantic Plume, a 160,000 km² area of less saline water with high concentrations of suspended sediments (Grodsky *et al.*, 2014). This region is dominated by the largest contiguous area of mangrove forest in the world (Schaeffer-Novelli *et al.*, 1990), with a total area of approximately 10,717 km² (Kjerfve & Lacerda, 1993).

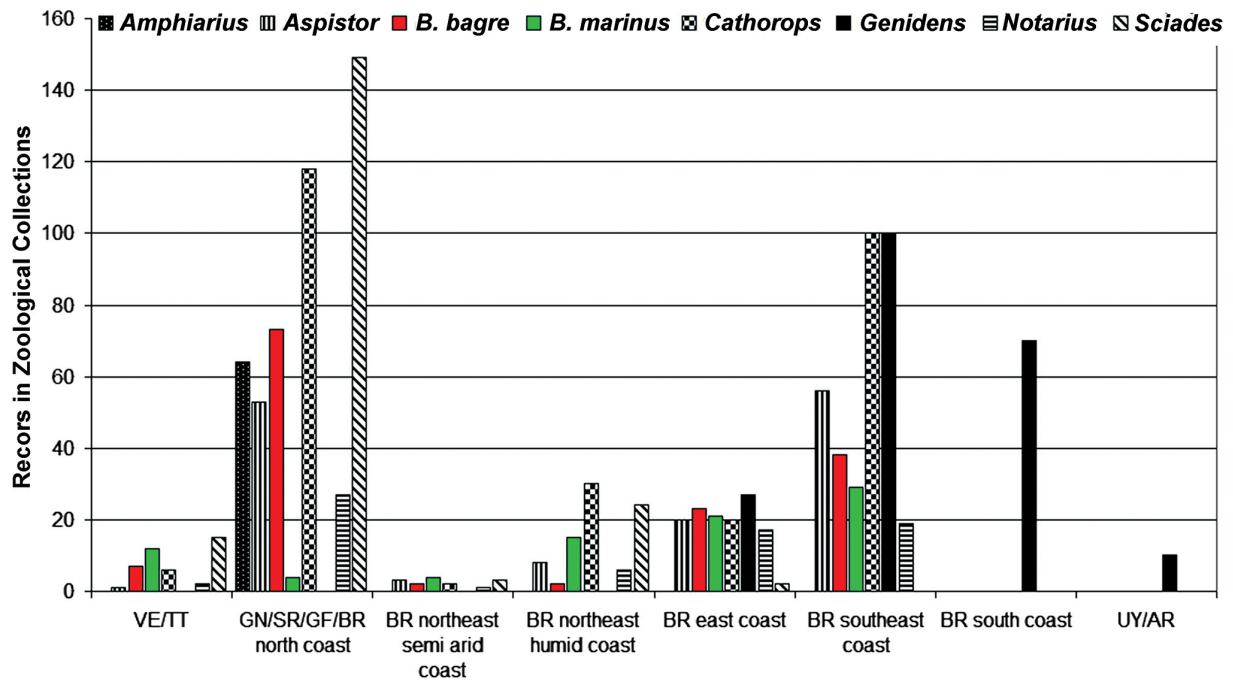


Fig. 7. A. Occurrence of *Bagre bagre*, *Bagre marinus* and other species of the genera *Amphiarius*, *Aspistor*, *Cathorops*, *Genidens*, *Notarius*, and *Sciades*, on the Atlantic coast of South America. Numbers of the lots deposited in zoological collections.

Table 4. Mean discharge of the hydrographic basins of the different coastal regions of Brazil, based ANA (2005).

Geographic areas	Hydrographic basin	Mean discharge
Northern coast	Amazon	131,947 m ³ /s
	Tocantins-Araguaia	13,624 m ³ /s
	Western Northeast	2,683 m ³ /s
Northern/ Northeast semiarid and humid Northeast coast	Parnaíba	763 m ³ /s
Eastern coast	Eastern Northeast	779 m ³ /s
	São Francisco	2,850 m ³ /s
Southeast coast	Eastern Atlantic	1,492 m ³ /s
	Southeast Atlantic	3,179 m ³ /s
	South Atlantic	4,174 m ³ /s
South coast	Paraná	11,453 m ³ /s
	Paraguay	2,368 m ³ /s
	Uruguay	4,121 m ³ /s

By contrast, the coast of northeastern Brazil is characterized by the presence of small- to medium-sized estuaries influenced by semiarid climates, with a discreet input of freshwater from the local rivers and a reduced discharge of sediments into the coastal waters (Ekau & Knoppers, 1999), and a restricted development of estuarine mangrove systems (Kjerfve & Lacerda, 1993). One exception is the São Francisco River, the second largest drainage basin in Brazil. The mouth of the São Francisco lies in the direct path of the Southern Equatorial Current (SEC), which intrudes onto the continental shelf and the coast at three points 7° S and 17° S, where Brazil's western boundary currents originate. Between 7° S and 10° S, the SEC flows northwestward, forming the Northern Brazil Current, and at around 15° S, it forms the Brazilian Current (Stramma *et al.*, 1990; Knoppers *et al.*, 2005).

The sediment plume emanating from the mouth of the São Francisco, which is deflected southwards (Knoppers *et al.*, 2005) inhibits the development of coral reefs because of the turbidity of the water off the coast of Sergipe and northern Bahia (Villwock *et al.*, 2005). The absence of coral reefs off the coast allows the tide to penetrate the local estuaries, contributing to the establishment of the region's mangroves which are fundamental to the life cycle of the ariids. Seasonal variations in the plume of the São Francisco, and the Northern Brazilian Current (Knoppers *et al.*, 2005), may contribute to the records of the species further north.

One other important factor related to the presence of areas of mangrove is the variation in the timing and intensity of the rainy season, which determined the breeding season of the ariid species (Araújo, 1988). Ariids are mouth-brooding fish, and the male carries a few large eggs in its mouth for approximately two months until the eggs hatch and the fry become free-swimming. The breeding season of ariids is related to the rainy season, extending into the dry season (Mishima & Tanji, 1983; Gomes & Araújo, 2004). On the northern coast of Brazil, the dry season ends in December, with the rainy season extending from January to June. On the northeastern coast, the late dry season is in February, with the early rainy season from March to May, and the late rainy season from June to August. On the southeastern coast, the dry season ends in October, and the rainy season lasts until March.

The environmental and behavioral variables identified on the coast of northeastern Brazil can be considered to be “soft” barriers, but even so, have a strong influence on the distribution of the two lineages of *B. bagre*, as well as all other ariid species found on the Brazilian coast. This conclusion is reinforced by the distribution of the species of other genera found on the Brazilian coast. *Amphiarus phrygiatus* (Valenciennes, 1840), *A. rugispinis* (Valenciennes, 1840), *Aspistor quadriscutis* (Valenciennes, 1840), *Cathorops arenatus* (Valenciennes, 1840), *Sciades parkeri* (Traill, 1832), *S. passany* (Valenciennes, 1840), and *S. couma* (Valenciennes, 1840) are all common throughout all area influenced by the plume of the Amazon-Orinoco, reaching as far south as the Parnaíba River (3° S) in Maranhão, which marks the southern/eastern limit of the northern coast of Brazil (Fig. 1). By contrast, the species of the genus *Genidens* (see Marceniuk & Menezes, 2007), which are common on the eastern and southern coasts of Brazil, are found no further north than Todos os Santos Bay (13° S) in Bahia (Marceniuk & Menezes, 2007), the northern limit of the east coast (Fig. 1).

Three other species that are common on the northern coast, *Sciades proops* (Valenciennes, 1840), *Notarius grandicassis* (Valenciennes, 1840), two marine species, and *Cathorops agassizii* (Eigenmann & Eigenmann, 1888), a freshwater species, are also represented by sister species in northeast, east and southeastern Brazil, with the same area of distribution for *Aspistor luniscutis*, sister

species of *A. quadriscutis*, restricted to the plume of the Amazon-Orinoco. Exceptions to the general pattern are the preferentially estuarine species of the genera *Cathorops*, that is, *C. spixii* (Agassiz, 1829) and *Sciades*, *i.e.*, *S. herzbergii* (Bloch, 1794), which are estuarine species found on both the northern and northeastern coasts of Brazil.

Implications for taxonomy and conservation. *Bagre bagre* is an important fishery resource in northern Brazil (Pinheiro & Fredou 2004; Isaac-Nahum, 2006), as well as the Guianas and Suriname (Le Bail *et al.*, 2000; Artigas *et al.*, 2003), but is rare or absent from commercial catches on the eastern and southeastern coast of Brazil (Freitas Netto *et al.*, 2002; Lopes *et al.*, 2002). The adequate definition of a species is a decisive step in the evaluation of its conservation status, and the development of effective management strategies (Marceniuk *et al.*, 2015).

The complete segregation of the two lineages found in the present study supports two distinct species of *Bagre bagre*. As Linnaeus proposed *Silurus bagre* for America meridionali (locality probably erroneous) without designating a holotype, it is impossible to determine which of the two forms this name applies to, requiring the designation of a neotype (ICZN, 1999), defining which of the two forms the name should be applied to, while the other population can be named based on one of the nominal species available: *Felichthys filamentosus* Swainson, 1839 (type locality - estuaries and rivers near Pernambuco), *Galeichthys gronovii* Valenciennes, 1840 (type locality - Terre-Ferme or Guyane, Maracaibo, Mana River Cayenne or Bahia), *Bagrus macronemus* Ranzani, 1841 (type locality - Brazil), and *Mystus carolinensis* Gronow, 1854 (type locality - Tropical America). The taxonomic framework, which defines the specific status of these lineages, will be discussed in a future paper.

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Appendix 1. Genbank accession number of haplotypes generated in the present study.

	Sample code	Shared haplotypes	Locality	GeneBank Accession Number
<i>B. bagre</i>	Bbag012 (H 1)	Bbag014, Bbag016, Bbag017, Bbag033, Bbag035, Bbag036, Bbag042, Bbag047, Bbag129, Bbag133, Bbag136, Bbag137, Bbag138, Bbag140, Bbag150, Bbag151, Bbag153, Bbag216, Bbag234, Bbag235, Bbag185, Bbag187, Bbag189, Bbag194, Bbag203, Bbag206, Bbag164, Bbag171, Bbag174, Bbag175, Bbag176, Bbag177, Bbag178, Bbag181.	Macapá, AP, Bragança, PA, São Luis, MA, Fortaleza, CE.	ATPase 8 and 6/ KU668591
<i>B. bagre</i>	Bbag025 (H2)	Bbag026, Bbag077, Bbag218, Bbag232, Bbag122, Bbag190, Bbag195, Bbag202, Bbag204, Bbag236, Bbag167, Bbag172, Bbag179.	Macapá, AP, São Luis, MA, Fortaleza, CE.	ATPase 8 and 6/ KU668592
<i>B. bagre</i>	Bbag028 (H3)	Bbag131, Bbag155, Bbag193.	Bragança, PA, São Luis, MA.	ATPase 8 and 6/ KU668593
<i>B. bagre</i>	Bbag126 (H4)	Bbag126.	Bragança, PA.	ATPase 8 and 6/ KU668594
<i>B. bagre</i>	Bbag132 (H5)	Bbag165, Bbag168.	Fortaleza, CE.	ATPase 8 and 6/ KU668595
<i>B. bagre</i>	Bbag136 (H 6)	Bbag136.	Bragança, PA.	ATPase 8 and 6/ KU668596
<i>B. bagre</i>	Bbag229 (H 7)	Bbag229.	Bragança, PA.	ATPase 8 and 6/ KU668597
<i>B. bagre</i>	Bbag120 (H 8)	Bbag121, Bbag163.	São Luis, MA, Fortaleza, CE.	ATPase 8 and 6/ KU668598
<i>B. bagre</i>	Bbag237 (H 9)	Bbag237.	São Luis, MA.	ATPase 8 and 6/ KU668599
<i>B. bagre</i>	Bbag166 (H 10)	Bbag166.	Fortaleza, CE.	ATPase 8 and 6/ KU668600
<i>B. bagre</i>	Bbag250 (H 11)	Bbag251, Bbag252, Bbag254, Bbag256, Bbag257, Bbag258, Bbag259, Bbag260, Bbag262, Bbag264, Bbag265, Bbag267, Bbag268, Bbag269.	Santos, SP.	ATPase 8 and 6/ KU668601
<i>B. bagre</i>	Bbag266 (H 12)	Bbag266.	Santos, SP.	ATPase 8 and 6/ KU668602
<i>B. bagre</i>	Bbag012 (H 1)	Bbag016, Bbag017, Bbag033, Bbag 35, Bbag 36, Bbag 47 - AP); (Bbag 129, Bbag 131, Bbag 133, Bbag 134, Bbag 136, Bbag 137, Bbag 138, Bbag 150, Bbag 151, Bbag 153, Bbag 216, Bbag 218, Bbag 234 - PA); (Bbag 120, Bbag 185, Bbag 187, Bbag 189, Bbag 194, Bbag 203, Bbag 206, Bbag 237 - MA); (Bbag 164, Bbag 166, Bbag 171, Bbag 174, Bbag 175, Bbag 176, Bbag 177, Bbag 178 - CE)	Macapá, AP, Bragança, PA, São Luis, MA, Fortaleza, CE.	Cytb/KU668603
<i>B. bagre</i>	Bbag014 (H 2)	Bbag014.	Macapá, AP.	Cytb/KU668604
<i>B. bagre</i>	Bbag025 (H 3)	Bbag025.	Macapá, AP.	Cytb/KU668605
<i>B. bagre</i>	Bbag026 (H 4)	Bbag026.	Macapá, AP.	Cytb/KU668606
<i>B. bagre</i>	Bbag028 (H 5)	Bbag122, Bbag193.	São Luis, MA.	Cytb/KU668607
<i>B. bagre</i>	Bbag042 (H 6)	Bbag042.	Macapá, AP.	Cytb/KU668608
<i>B. bagre</i>	Bbag077 (H 7)	Bbag126, Bbag190, Bbag 202, Bbag 236, Bbag167, Bbag172, Bbag179.	Bragança, PA, São Luis, MA, Fortaleza, CE.	Cytb/KU668609
<i>B. bagre</i>	Bbag132 (H 8)	Bbag165, Bbag168.	Fortaleza, CE.	Cytb/KU668610
<i>B. bagre</i>	Bbag140 (H 9)	Bbag155.	Bragança, PA,	Cytb/KU668611
<i>B. bagre</i>	Bbag229 (H 10)	Bbag121, Bbag163.	São Luis, MA, Fortaleza, CE.	Cytb/KU668612
<i>B. bagre</i>	Bbag232 (H 11)	Bbag204, Bbag181.	São Luis, MA, Fortaleza, CE.	Cytb/KU668613
<i>B. bagre</i>	Bbag235 (H 12)	Bbag235.	Bragança, PA.	Cytb/KU668614
<i>B. bagre</i>	Bbag195 (H 13)	Bbag195.	São Luis, MA.	Cytb/KU668615
<i>B. bagre</i>	Bbag250 (H 14)	Bbag251, Bbag252, Bbag254, Bbag257, Bbag258, Bbag259, Bbag260, Bbag 262, Bbag 264, Bbag 266, Bbag 267, Bbag 268, Bbag 269.	Santos, SP.	Cytb/KU668616
<i>B. bagre</i>	Bbag256 (H 15)	Bbag256	Santos, SP.	Cytb/KU668617
<i>B. bagre</i>	Bbag265 (H 16)	Bbag265.	Santos, SP.	Cytb/KU668618
<i>B. marinus</i>	Bma02 (H 1)	Bma02, Bma03	Fortaleza, CE.	ATPase 8 and 6/ KU730294
<i>B. marinus</i>	Bma02 (H 1)	Bma02, Bma03	Fortaleza, CE.	Cytb/KU720393

Appendix 2. Molecular sequences from GenBank.

Species	Tissue Number	Locality	GeneBank Accession Number (Cytb/AtPase 8/6)
<i>Gogo arcuatus</i>	Garc		FJ013160/FJ013144
<i>Cathrops mapale</i>	Cmapa		GQ982422/GQ982456
<i>Galeichthys sp.</i>	Gsp		FJ013163/FJ013147
<i>Cathrops dasycephalus</i>	Cdasy		DQ990467/DQ990639
<i>Cathrops fuerthii</i>	Cfuer		DQ990469/DQ990641
<i>Galeichthys peruvianus</i>	Gperu		DQ990462/DQ990634
<i>Galeichthys ater</i>	Gater		FJ013261/FJ013145
<i>Galeichthys feliceps</i>	Gfeli		FJ013175/FJ013146
<i>Bagre bagre</i>	Bbag	Orenoco River, Venezuela	AY688673/DQ990678
<i>Bagre marinus</i>	Bmar		AJ581355/DQ990679
<i>Bagre aff. marinus</i>	Baffmar		DQ990500/DQ990680
<i>Bagre pinnimaculatus</i>	Bpinn		AJ580998/DQ990677
<i>Bagre panamensis</i>	Bpana		AJ580999/DQ990676