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DNA barcoding of coastal ichthyofauna from Bahia, northeastern Brazil, South Atlantic: High efficiency for systematics and identification of cryptic diversity

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ABSTRACT

In spite of the utility of DNA barcoding in discriminating marine fish species, few studies using this technique are available along the Brazilian coast, which encompasses nearly 9000 km, distinct biogeographic regions and endemism rates. Therefore, in the present work, we provided the first DNA barcode library based on sequencing of the mitochondrial gene cytochrome c oxidase subunit I (COI) for Actinopterygii fish from northeastern coast of Brazil. The database included 459 specimens from 54 genera, 31 families and 13 orders, with mean interspecific genetic distance of and mean intraspecific distance of 0.15%. As a result, the neighbor-joining (NJ) tree resulted in the molecular identification of 78 taxa. Cryptic species were indicated in *Eucinostomus* (mojarras) since the 82 specimens morphologically identified as *Eucinostomus melanopterus* or *Eucinostomus* sp. formed five clusters with intraspecific genetic distances up to 0.31% and lowest interspecific distance of 11.14%. *Eucinostomus jonesii* and *Eucinostomus harengulus* were identified for the first time in ichthyofauna inventories from northeastern Brazil. The present work has also added new records to the Barcode of Life Data System (BOLD), thereby favoring the identification of other specimens at different life stages or in forensic studies. The present dataset reinforces the reliability of DNA barcoding to estimate the overlooked richness of marine fish in Brazilian coast with application in conservation, ecology, systematics and fisheries.

1. Introduction

The utilization of a small fragment of the mitochondrial gene cytochrome c oxidase subunit I (COI) in DNA barcoding represented a breakthrough in the molecular identification of animal species (Hebert et al., 2003). This approach has early proved to be successful in taxonomy and biodiversity studies of marine organisms (Ward et al., 2009; Zemlak et al., 2009). The

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creation of a large database of DNA barcodes from marine species is also advantageous to identify larval stages that could not be assigned by traditional morphology and to forensic studies in commercial fisheries (Bhattacharya et al., 2015). Indeed, two global campaigns have been launched to provide a library of DNA barcodes for fish worldwide, named FISH-BOL (http://www.fishbol.org) and SHARK-BOL (http://www.sharkbol.org), comprising nearly 11 thousand freshwater and marine species so far.

Likewise, DNA barcoding in Neotropical freshwater ichthyofauna has been highly efficient to reveal cryptic and/or unknown species with identification rates at species level higher than 98% (e.g., Pereira et al., 2013; Gomes et al., 2015). These studies show the applicability of DNA barcoding to both systematics and ecology, being helpful to establish priority areas for conservation based on endemism rates.

On the other hand, studies of DNA barcodes in marine fish from South America are still scarce (Mabragaña et al., 2011). In Brazil, the country with the largest coastline in Southwestern Atlantic (about 9000 km), DNA barcode libraries are available only for fish species from São Paulo coast, southeastern region (Ribeiro et al., 2012). Other reports of COI analyses are scattered and focused on the identification of fish products in Brazilian fisheries, including frauds (Brito et al., 2015) and commercialization of endangered species (Palmeira et al., 2013).

Furthermore, distinctive biogeographic features along the Brazilian coast account for an unexpected differentiation of fish fauna between apparent continuous regions. Several studies have demonstrated that the Southern portion of central coast in Brazil (between the states of Bahia and Espírito Santo) is an important zoogeographic transition zone (Martins et al., 2007). Natural geographic barriers in this zone, such as Abrolhos bank and Vitória-Trindade Seamount Chain, cause changes in current flow (Campos et al., 2000), determining distinct environmental conditions that could explain local adaptations and differences in fish assemblages.

In fact, marine fish species with restricted range along Northern/Northeastern or Southeastern/Southern coasts of Brazil have been described over the last decades (e.g. Carvalho Filho and Ferreira, 2013). In weakfish (genus *Macrodon*), genetic and morphometric analyses showed that *Macrodon ancylodon* actually comprised two isolated clades, each one specific to tropical and subtropical regions in Brazil, being the latter validated as *Macrodon atricauda* (Santos et al., 2006; Carvalho-Filho et al., 2010). In other cases, endemic species were identified along the Brazilian province, but distinguished from the Caribbean sister taxa, like *Halichoeres dimidiatus* and *Halichoeres penrosei*, formerly considered synonyms of *H. cyanocephalus* and *H. maculipinna*, respectively (Rocha, 2004).

In spite of this peculiar distribution pattern of marine ichthyofauna in Brazil and the potential of DNA barcoding in uncovering fish diversity, the richness and endemism levels of northeastern Brazilian coast remains poorly studied by this approach, even though this region encompasses more than 2000 km of coastline. Thus, the goal of the present work was to provide the first extensive COI library of marine fish from Northeastern Brazil. For that, fish collection was performed along the coast of the state of Bahia, because of its extension and diversity of habitats, including two of the largest estuaries in Brazil (Todos os Santos and Camamu bays).

2. Material and methods

The fish specimens were collected from 2009 to 2014 in four localities along the coast of Bahia, northeastern Brazil: Todos os Santos Bay, Tinharé Island, Camamu Bay, and Olivença (Fig. 1). Most specimens were caught by local fisherman using commercial trawl nets, but gill nets, seine nets, and snorkeling were also used in some cases, particularly close to reef areas. The fish collection was authorized by the Chico Mendes Institute of Biodiversity Conservation (ICMBio) on behalf of Paulo Roberto Antunes de Mello Affonso and Iracilda Sampaio (licenses 31360–1 and 27027–2).

All specimens were photographed, morphologically identified, and deposited in the fish collection at Universidade Estadual do Sudoeste da Bahia. A fragment of muscle tissue was removed from each specimen and stored in ethanol 96% at −20 °C. Total DNA was extracted from muscle tissues using the Wizard Genomic DNA Purification kit (Promega), according to the manufacturer's instructions.

The barcode sequences were based on a fragment of nearly 650 bp from the 5' portion of COI amplified via PCR using the primer cocktail C_FishF1t1–C_FishR1t1 (Ivanova et al., 2007). Each PCR comprised 0.2 mM of dNTPs, buffer 1×, 2 mM of $MgCl_2$, 0.4 μM of each primer, 1 U of Taq polymerase, 50–100 ng of template DNA and ultrapure water to a final volume of 15 μl. The PCRs were carried out in a Veriti thermocycler (Applied Biosystems) using the following program: 5 min at 95 °C; 35 cycles of 40 s at 94 °C, 40 s at 55 °C and 1 min at 72 °C, and a final extension step at 72 °C for 10 min.

The amplification of COI fragments was confirmed by electrophoresis in 1% agarose gel, using 3 μl of each PCR product stained with bromophenol blue and Gel Red®. Afterward, the samples were purified in 20% polyethylenoglicol.

The purified fragments were sequenced bidirectionally using the BigDye Terminator v3.1 Cycle Sequencing Ready Reaction kit (Applied Biosystems). This reaction comprised 4.75 μl of ultrapure water, 1.5 μl of buffer 5×, 0.75 μl of BigDye terminator mix, 2 μl of M13F or M13R primers (0.8 pmol) and 1 μl of purified PCR product. The conditions used were 1 min at 96 °C; 35 cycles of 15 s at 96 °C, 15 s at 50 °C and 2 min at 60 °C. After precipitation in EDTA 125 mM, 100% and 80% ethanol, the samples were sequenced automatically using an ABI 3500XL Genetic Analyzer sequencer (Applied Biosystems).

The contigs were obtained from forward and reverse COI sequences using the software DNA Baser Sequence Assembler v4 (www.DnaBaser.com). The multiple sequence alignment was obtained via Clustal W Multiple Alignment available in the software BioEdit Sequence Alignment Editor 7.1.9 (Hall, 1999). The alignment was exported to the software MEGA v.6 (Tamura et al., 2013) to provide a NJ tree (Saitou and Nei, 1987) based on Kimura-2-parameter (K2P) substitution model (Kimura, 1980) and the confidence of branches was verified by bootstrap (1000 replicates) (Felsenstein, 1985).