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Range distribution and contributions to taxonomy of *Elops smithi* (ELOPIFORMES: ELOPIDAE)

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Abstract: This study presents the first record of *Elops smithi* for northern Brazil. The evidence suggests this species is being misidentified incorrectly as *Elops saurus* in estuaries of the Western Atlantic Ocean. Here, morphological, molecular, and cytogenetic evidence identified all ladyfish specimens from one estuary in the region as *E. smithi*. Thus, at least *Elops smithi* occurs in the northern coast of Brazil and it is recommended that specimens from this region identified as *E. saurus* be further investigated with genetic and cytogenetic tools in order to assure a correct species identification.

Key words: Elopidae, Karyotype, Ladyfish, Malacho, Mitochondrial DNA.

INTRODUCTION

The family Elopidae includes only the genus *Elops*, with seven species distributed throughout the tropics. Until recently, the ladyfish, *Elops saurus*, was the only elopid species known to occur in the northwestern Atlantic (Obermiller and Pfeiler 2003, McBride et al. 2010). However, Smith (1989) identified the presence of two sympatric larval morphs within this area, distinguished by the number of myomeres. Based on the analysis of mitochondrial *Cytb* sequences and morphological data, McBride et al. (2010) confirmed that the morph with the lower myomere count was, in fact, a distinct species, which they denominated *Elops smithi*. The divergence of the *Cytb* haplotypes of *E. saurus* and *E. smithi* ranged from 2.3% to 2.9% (McBride et al. 2010).

Elops saurus is distributed throughout the northwestern Atlantic, including the Gulf of Mexico and the Yucatan Peninsula, and was

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originally considered to inhabit only the Northern Hemisphere, while *E. smithi* occurs in the central Atlantic, Bahamas, and the Caribbean Sea, and is sympatric with *E. saurus* on the southeastern coast of the United States and in the Gulf of Mexico (McBride and Horodysky 2004, McBride et al. 2010).

In the southwestern Atlantic, *E. smithi* has been recorded in marine, coastal, and estuarine environments (Lucena and Carvalho-Neto 2012, Machado et al. 2012, Sánchez-Botero et al. 2016). In most surveys of fish populations on the Brazilian coast, however, *E. saurus* has been identified as the most common elopid species (Franco et al. 2014, Garcia-Junior et al. 2015, Pinheiro et al. 2015, Marceniuk et al. 2017, Medeiros et al. 2018, Mendes et al. 2018).

In studies of fish, a cytogenetic approach has become an increasingly effective tool for the identification of cryptic species (Harrison et al. 2007, Martinez et al. 2016), the elucidation of the origin of hybrids (Majtánová et al. 2016, Pereira et al. 2014), and the interpretation of phylogenetic relationships (Majtánová et al. 2017, Ramirez et al. 2017), as well as the understanding of chromosome structure (Jacobina et al. 2011). Until now, the only cytogenetic data available for the family Elopidae refer to the diploid number of *E. saurus* (Doucette and Fitzsimons 1982). The present study provides the first records of *E. smithi* from the region of the Brazilian Amazon estuary and discusses the relative effectiveness of morphological-meristic, molecular, and cytogenetic data approaches for the identification of the species.

MATERIALS AND METHODS

COLLECTION OF *Elops smithi* SPECIMENS

Larval, juvenile, and adult *Elops* specimens were collected for this study. The larvae were collected from the subsurface layer (≈ 0.5 m depth) of the water column in the Taperaçu estuary in northern Brazil (46°45'25.2" W, 0°56'47.1" S; Fig. 1, zone 1) with horizontal plankton trawls with conical nets with mesh of 300 μm and 500 μm. Once collected, some *E. smithi* larvae were immediately stored in 70% alcohol for molecular analyses, while others were stored in 4% formaldehyde for meristic analyses. The larvae were identified based on the literature (Bonecker and Castro 2006, McBride et al. 2010), and described following the general criteria and terminology proposed by Gehringer (1959).

Ten juveniles were collected from a saline lagoon located in a tract of mangrove near the Taperaçu estuary (46°40'5.71" W, 0°53'53.22" S; Fig. 1, zone 2) using a fishing net with a 25 mm mesh. The adult specimen was captured on the adjacent continental shelf (46°35'24.0" W, 0°49'32.0" S; Fig. 1, zone 3) using the same net. All specimens were deposited in the ichthyological collection of the Ichthyology Laboratory of the Aquatic Ecology Group at the Federal University of Pará in Belém (GEA.ICT).

DNA EXTRACTION, PCR, AND SEQUENCING

To confirm the identification of the species, DNA was extracted from the specimens based on the standard protocol for the DNA Wizard Genomic Purification kit (Promega Corporation, Madison, WI - USA). A fragment of the mitochondrial Cytochrome *b* (*Cytb*) gene was amplified by PCR, using the primers Cyb-09H (Song et al. 1998) and Cyb-07L (Taberlet et al. 1992), using the protocol described by McBride et al. (2010). The amplified products were purified using the ExoSAP-IT enzyme and sequenced in an ABI 3500 automatic sequencer (Applied Biosystems) using a Big-Dye Terminator Cycle Sequencing kit (Applied Biosystems).

MOLECULAR ANALYSES

The *Cytb* sequences were amplified to investigate the genetic differences and similarities between

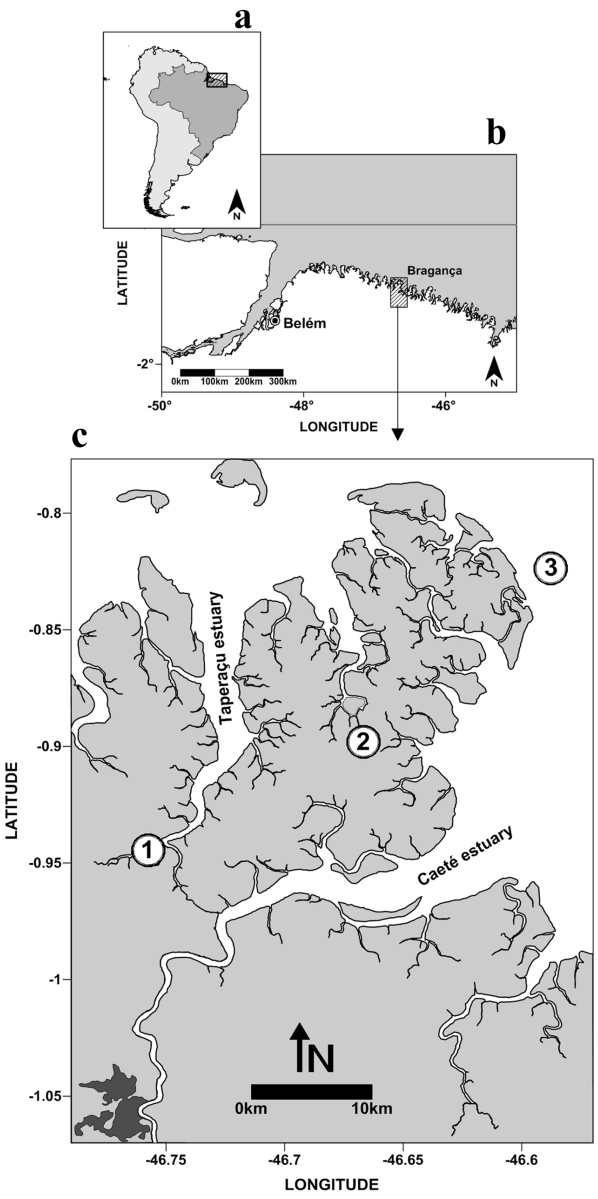

Figure 1 - Study area: South America (**a**), Northern Brazil coast (**b**), and positions of the sampling stations in Pará coastal zone - 1, Taperaçu estuary; 2, salt lagoon; 3, continental shelf (**c**).

our specimens and the elopid species found in the southwestern Atlantic (*E. saurus* and *E. smithi*) and eastern Pacific (*Elops affinis*). The osteoglossid *Osteoglossum bicirhosum* (Vandelli 1829) was used as the outgroup. Genetic divergence was determined based on the Kimura 2-parameter statistic (K2P), and a neighbor-joining distance tree (bootstrap with 1,000 pseudo-replicates) of the *Elops* species was constructed in MEGA version 7 (Kumar et al. 2016).

CYTOGENETIC ANALYSES

Fragments of fin were removed from the specimens in the laboratory. Cell culture followed the protocol of Sasaki et al. (1968), and mitotic chromosomes were obtained from a fixed and hypotonized cell suspension by the air-drying procedure (Bertollo et al. 1978). The chromosomes were NOR-banded to reveal the active nucleolus organizer regions, following the procedure developed by Howell and Black (1980). The banded metaphases were visualized and photographed under a LEICA 1000 DM microscope, in a light field with an immersion lens. The karyotype of the species was assembled using GENASIS version 7.2.6.19509.

MORPHOLOGICAL ANALYSES

The reliability of the diagnostic characteristics described in previous studies was assessed by comparing the morphological-meristic traits (standard length [SL], number of dorsal, anal, and pectoral rays, total number of myomeres, and number of pre-anal myomeres) of the *Elops* specimens collected in the present study with the parameters described in the literature.

RESULTS

MORPHOLOGICAL AND MOLECULAR IDENTIFICATION OF THE *Elops smithi* LARVAE

A total of 59 *E. smithi* larvae were identified in the plankton samples collected from the Taperaçu estuary. The shortest standard length of a larva was 22.99 mm, and the longest, 34.95 mm, with a mean of 29.27±4.81 mm. The most effective morphological character for the identification of *E. smithi* was the pre-anal myomere count. The larvae collected in the Taperaçu estuary had 61–67 pre-anal myomeres.

The larvae represented two development stages, the leptocephalus (64.40% of the larvae) and pre-metamorphic (35.60%) stages. The juveniles and the only adult specimen presented 21–22 dorsal fin rays, 14 anal fin rays and 13–14 pectoral fin rays.

In the present study the *E. smithi* larvae were captured in a saline estuary, which is consistent with the data on the larval ecology of *E. saurus*. The juveniles (SL: 175–180 mm) were also collected in a coastal saline lagoon near the Taperaçu estuary. The adult specimen (SL: 470 mm) was collected at sea, 41.8 Km off the coast of Pará.

Comparisons of the *Cytb* sequences with those of the *Elops* species available in GenBank indicated that all the specimens collected on the coast of Pará belonged to the species *E. smithi* (Fig. 2A). This diagnosis was further reinforced by the mean genetic distance of 0.8% within the group that includes the specimens collected during the present study and *E. smithi* from other areas in the coastal western Atlantic (Table I).

TABLE I
Summary of the pairwise sequence divergence (K2P percentage), based on *Cytb* gene, between *Elops* species.

		1	2	3	4
1	*Elops smithi*_this study				
2	*Elops* sp.	0.008			
3	*Elops saurus*	0.019	0.021		
4	*Elops affinis*	0.029	0.028	0.032	

CYTOGENETIC ANALYSES

Fifty metaphases were analyzed to describe the diploid number, and 30 were used to describe the NOR banding. The diploid number of *E. smithi* was 2n = 50 (6M + 4ST + 42A), with a fundamental number (FN) of 60 (Fig. 2b). The NORs were observed in the centromere of pair 14, presenting different sizes for the banding signals.

DISCUSSION

The present study is the first to record *E. smithi* (larvae, juveniles and adult specimens) on the Amazon coast of northern Brazil (Fig.1). The majority of the *E. smithi* larvae analyzed here had 61–67 pre-anal myomeres, which is comparable with the counts recorded for *E. smithi* in Uruguay (Lucena and Carvalho-Neto 2012) and southern Brazil (Machado et al. 2012). The genetic distances recorded in the *Cytb* sequences (1.9–2.9%) also distinguished *E. smithi* unequivocally from *E. saurus*.

The leptocephali and pre-metamorphic *E. smithi* larvae were collected in the innermost portion of the Taperaçu estuary, reinforcing the conclusion that this species is estuarine-dependent, as suggested by Ray (1997) and McBride et al. (2001). The life stages of *Elops* species are well-defined, with the adults and early larval stages being found on the continental shelf, while spawning occurs in coastal waters and the later larval and juvenile stages are found in estuaries (Gehringer 1959, Eldred and Lyons 1966, Adams et al. 2014).

The karyotype of *E. smithi*, described here for the first time, is clearly different from that of *E. saurus* – 2n = 48 (6M/ST + 42ST/A, FN = 54) – which was described by Doucette and Fitzsimons (1982). In *E. smithi*, not only is the diploid number different, but the whole arrangement is quite distinct – 2n = 50 (6M + 4ST + 40A, NF = 60). These differences provide diagnostic markers for the distinction of the two species, reinforcing the conclusions of Harrison et al. (2007) and Sczepanski et al. (2010) on the importance of these markers for the description of new species of fish.

The *E. smithi* karyotype has a larger number of two-armed chromosomes than that of *E. saurus*. As the basal karyotype of marine teleosts is assumed to be 2n = 48 single-armed chromosomes (Vitturi et al. 1995, Nirchio et al. 2004), fusions and chromosomal rearrangements may have played an

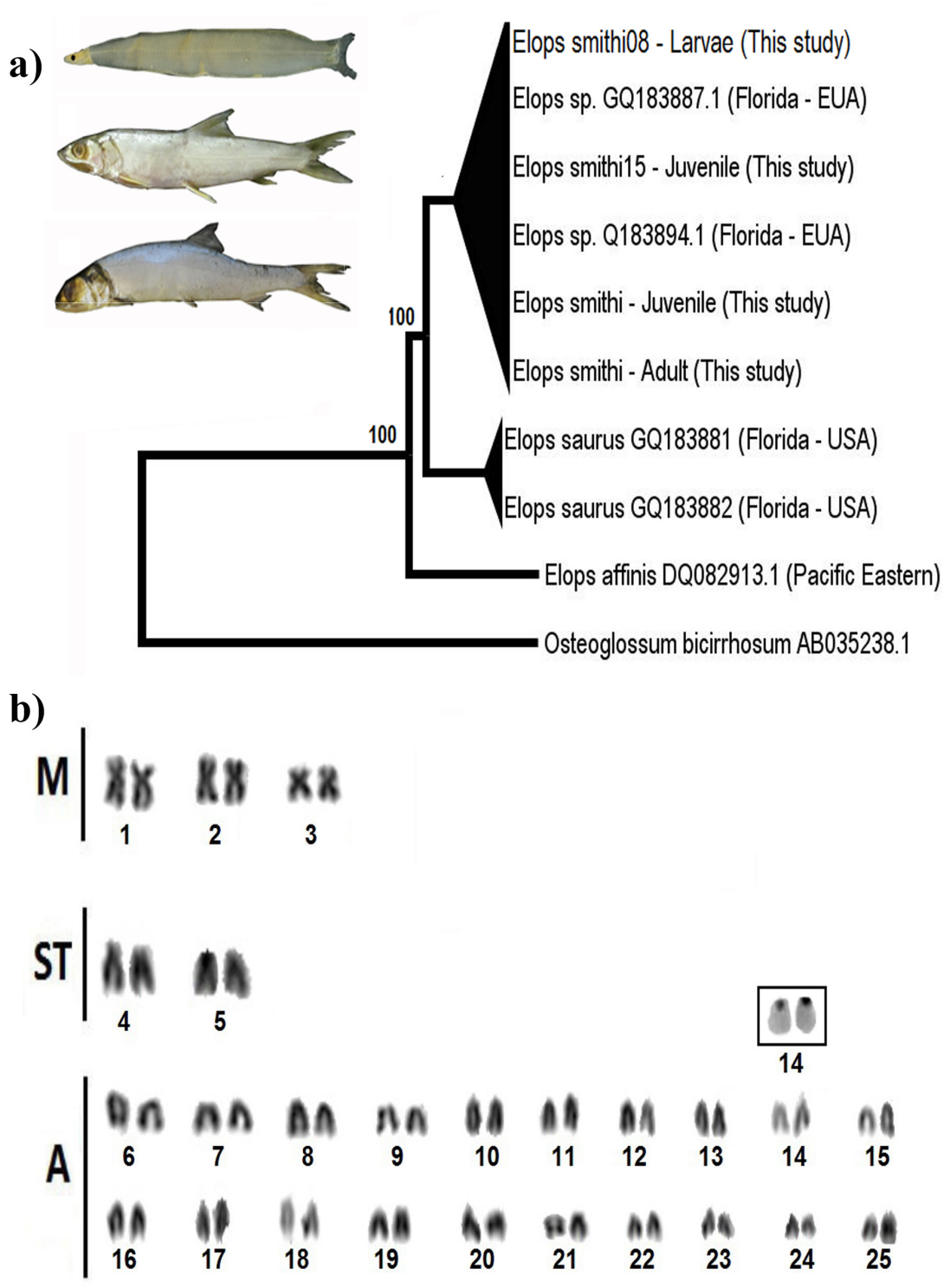

Figure 2 - a) Distance tree (neighbour-joining) with *Elops* species: pre-metamorphic larvae (33.31 mm SL), juvenile individual (175 mm SL), and adult individual (410 mm SL). The numbers at the branches are the bootstrap support values for K2P; **b**) Karyotype of *Elops smithi* stained conventionally with Giemsa and the nucleolar organizer region (NOR) bearing pair (14) after silver nitrate staining.

important role in the chromosomal evolution of the Elopiformes (Merlo et al. 2005).

This is the first description of NOR banding in an *Elops* species, datas of NOR has providing important insights to taxonomy of some groups of fish (Benzaquem et al. 2008). In vertebrates, NORs are associated predominantly with the telomeric and interstitial regions of the chromosome, but in *E. smithi*, these markers were found in the centromeric region, a pattern rarely observed in fish, which has been interpreted as the result of paracentric inversions or translocations (Bittencourt et al. 2014). Variations in the size of the NORs in fishes are associated primarily with structural changes, such as duplications and deletions (Cross et al. 2006, Fujiwara et al. 2007). Such events may have occurred during karyotypical evolution of *E. smithi*.

The findings of this study indicate clearly that the morphological similarities of *E. saurus* and *E. smithi* may have led to the frequent misidentification of the specimens collected in past surveys. Given this, existing specimens from the northern Brazilian coast identified as *E. saurus* require new genetic or cytogenetic confirmation, given that sympatry of these two species on the western coast of the South Atlantic is yet to be confirmed.

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AUTHOR CONTRIBUTIONS

RPCS and AGC wrote the manuscript and performed cytogenetic and molecular analysis; RPCS, DS and RMC collected samples and performed morphological identification; EHCO and GCSO provide the structural and technical support for cytogenetic analysis; MV and IS assisted in molecular analysis and provided financial support. All authors edited in approved the manuscript.

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