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Molecular cytogenetics insights in two pelagic big-game fishes in the Atlantic, the tarpon, *Megalops atlanticus* (Elopiformes: Megalopidae), and the sailfish, *Istiophorus platypterus* (Istiophoriformes: Istiophoridae)

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Some pelagic and usually large sized fishes are preferential targets for sport and commercial fishing. Despite their economic importance, cytogenetic data on their evolutionary processes and management are very deficient, especially due to logistical difficulties. Here, information for two of such charismatic species, the tarpon, *Megalops atlanticus* (Elopiformes: Megalopidae), and the sailfish, *Istiophorus platypterus* (Istiophoriformes: Istiophoridae), both with a wide Atlantic distribution, were provided. Cytogenetic data were obtained using conventional methods (Giemsa staining, Ag-NORs technique, and C-banding), base-specific fluorochrome staining and fluorescence *in situ* hybridization (FISH) with rDNA probes. *Megalops atlanticus* has 2n = 50 chromosomes, all acrocentric ones (NF = 50), while *Istiophorus platypterus* has 2n = 48 chromosomes, 2m + 2st + 44a (NF = 52). *Megalops atlanticus* populations from the South Atlantic and Caribbean share identical karyotypic patterns, likely associated with gene flow between them. In turn, *I. platypterus* presents karyotype similarities with phylogenetically close groups, such as Carangidae. The chromosomal characteristics of these species highlight their independent evolutionary paths. Additionally, the current data contribute to knowledge of new aspects of pelagic fish fauna and will support further comparative studies with congeneric species, clarifying evolutionary karyotype trends of these fish groups.

Keywords: Animal cytogenetics, Chromosome evolution, rDNA, Species conservation.

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Alguns peixes pelágicos de grande porte são alvos preferenciais para a pesca esportiva e comercial. Apesar de sua importância econômica, os dados citogenéticos sobre seus processos evolutivos e de manejo são muito deficientes, principalmente devido às dificuldades logísticas. Aqui são apresentadas informações cromossômicas de duas espécies carismáticas, o tarpão, *Megalops atlanticus* (Elopiformes: Megalopidae), e o agulhão-vela, *Istiophorus platypterus* (Istiophoriformes: Istiophoridae), ambos com ampla distribuição no oceano Atlântico. Os dados citogenéticos foram obtidos usando métodos convencionais (coloração em Giemsa, técnica de Ag-NORs e bandamento C), coloração com fluorocromos específicos e hibridização fluorescente *in situ* (FISH) com sondas DNAr. *Megalops atlanticus* possui 2n = 50 cromossomos, todos acrocêntricos (NF = 50), enquanto *Istiophorus platypterus* possui 2n = 48 cromossomos, 2m + 2st + 44a (NF = 52). Populações de *M. atlanticus* do Atlântico Sul e Caribe compartilham padrões cariotípicos idênticos, provavelmente associados ao fluxo gênico entre regiões. Por sua vez, *I. platypterus* apresenta semelhanças cariotípicas micro e macroestruturais com grupos filogeneticamente próximos, como Carangidae. As características cromossômicas destas espécies destacam seus caminhos evolutivos independentes. Adicionalmente, os dados apresentados contribuem com novos aspectos da fauna pelágica e apoiarão futuros estudos comparativos com espécies congenéricas, esclarecendo as tendências evolutivas do cariótipo destes grupos de peixes.

Palavras-chave: Citogenética animal, Conservação de espécies, DNAr, Evolução cromossômica.

INTRODUCTION

Pelagic ecosystems represent one of the largest environments on the planet and, in general, little is known about the evolutionary features of its ichthyofauna. Marine pelagic fishes can reach an extensive geographical distribution, a condition that has direct implications for their genetic and cytogenetic patterns (Galetti *et al*., 2000, 2006; Soares *et al*., 2013, 2017). However, cytogenetic analyses in large marine fishes, especially the pelagic ones, are very scarce even in those of great economic value, mostly due to logistical restrictions involved (Soares *et al*., 2013, 2014).

A large phylogenetic spectrum of fish groups inhabit the pelagic ecosystems, including representatives of the orders Elopiformes and Istiophoriformes. Elopiformes presents itself as a sister group to all the others groups of the superorder Elopomorpha (Chen *et al*., 2014) and comprises only two old and slightly diverse families, the Elopidae (with only the *Elops* genus, with 7 species) and Megalopidae (with only the *Megalops* genus, with 2 species), with an estimated origin of 215 Mya (Broughton *et al*., 2013). Elopiformes (9 spp.) is hundreds of times less diverse than other Elopomorpha groups, such as Anguilliformes (995 spp.) (Fricke *et al*., 2020). Therefore, due to their phylogenetically position and evolutionary aspects, the cytogenetic patterns of Elopiformes are one important element that contributes to clarify the karyotype evolution in Teleostei as a whole.

Istiophoriformes includes the families Istiophoridae and Xiphiidae, also comprising important species in sport fishing, such as the sailfish *I. platypterus* (Shaw, 1792), globally distributed throughout the world's tropical and subtropical marine water, and the swordfish *Xiphias gladius* Linnaeus, 1758 widely distributed in the Atlantic, Pacific and Indian Oceans (Fricke *et al*., 2020). The origin of the Istiophoriformes probably occurred around ~71 Mya, in the Late Cretaceous (100.5–66 Mya), and the diversification of istiophorids and swordfishes originated around ~17.5 Mya, in the Early Miocene (23–16 Mya) (Santini *et al*., 2013).

Sailfishes are active predators distributed in pelagic ecosystems in tropical and temperate regions, morphologically characterized by a protruding upper jaw (Nakamura, 1985), and considered to be among the fastest swimmers in the oceans (Svendsen *et al*., 2016). Despite their ecological and commercial importance, the global genetic population structure of sailfish is not well understood (Lu *et al*., 2015), and cytogenetic information on these fishes is still lacking.

In the present study we provide a detailed karyotypic analysis of the tarpon, *Megalops atlanticus* Valenciennes, 1847 (Elopiformes: Megalopidae) and the sailfish, *Istiophorus platypterus* (Istiophoriformes: Istiophoridae), both representatives of marine species with a high economic importance, especially in the lucrative sportfishing market (Ault, Luo, 2013; Adams *et al*., 2019). These species occupy vast tropical and subtropical oceanic regions, where *M. atlanticus* inhabits coastal waters, including estuaries and lagoons, and *I. platypterus* is eminently oceanic (Nakamura, 1985; Riede, 2004; Ault, 2010). It was applied conventional and molecular cytogenetic procedures (Giemsa, Ag- NORs, C- and MM/DAPI banding, and mapping of the 18S and 5S rDNAs, in order to investigate the chromosomal patterns of the current species, provide a first basis to further interpopulation comparisons, and highlight the main cytogenetic divergences between Elopifomes and Istiophoriformes groups.

MATERIAL AND METHODS

Samples. Five juvenile individuals of *Megalops atlanticus* (Elopiformes: Megalopidae) and four individuals (undetermined sex) of *Istiophorus platypterus* (Istiophoridae) were collected from the Brazilian Northeast coast, in the Rio Grande do Norte State (*M. atlanticus* and *I. platypterus* – 06°20'S 35°15'W) (Fig. 1), through sport and commercial fishing vessels. Collections had the authorization of the Chico Mendes Institute for Biodiversity Conservation (ICMBio), System of Authorization and Information about Biodiversity (SISBIO-Licenses Nº 19135–1, 131360–1 and 27027–2), a National System of Genetic Resource Management and Associated Traditional Knowledge (SISGEN). All cytogenetics procedures were performed at the Laboratory of Genetics of Marine Resources from the Federal University of Rio Grande do Norte.

Chromosome preparation, C-banding, Ag-NOR and MM/DAPI staining. Chromosome preparations were performed from kidney tissues dissociated in 9.5 ml RPMI 1640 medium with 0.2 ml colchicine, for 30 min, followed by hypotonization with KCl 0.075, for 25 min at room temperature (Gold *et al*., 1990). The cell suspension was dropped onto clean slides covered with a thin film of water at 60 ºC. After drying,

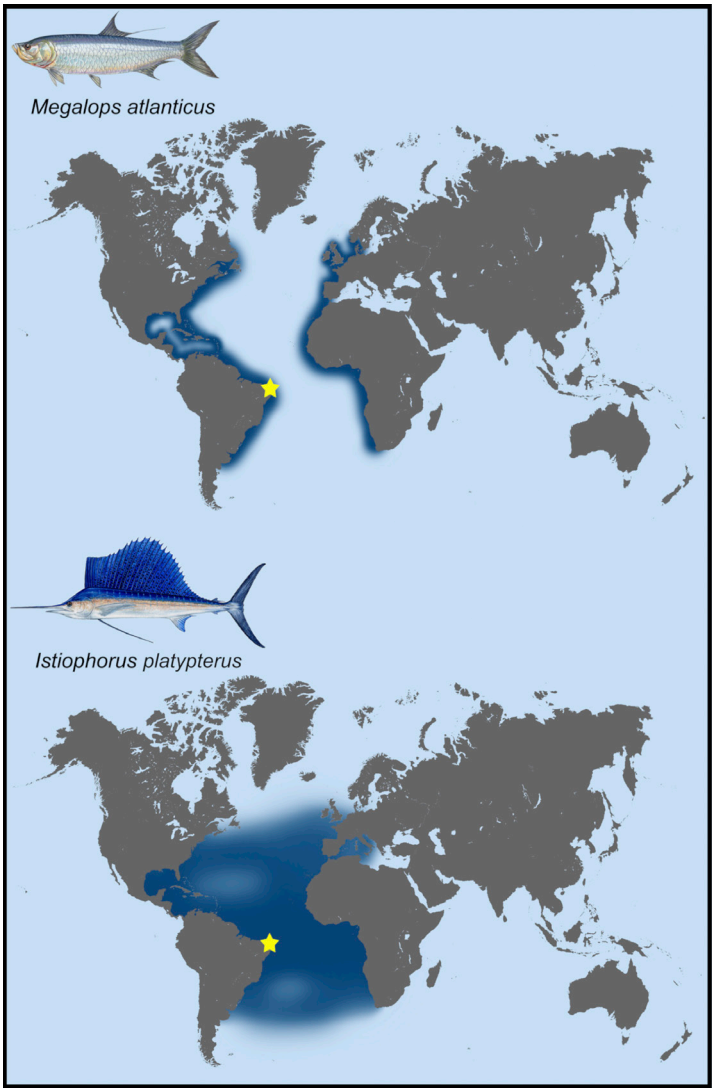

FIGURE 1 | Geographic distribution map of *Megalops atlanticus* (Megalopidae) and *Istiophorus platypterus* (Istiophoridae) across the Atlantic ocean. The shaded areas represents the occurrence and the yellow stars represent the collection sites of the species.

chromosomes were stained with Giemsa 10%, diluted in pH 6.8 phosphate buffer. Nucleolar organizing regions (NORs) and the constitutive heterochromatin were visualized by Silver nitrate staining (*i.e.*, Ag-NORs) and C-banding, according to Howell, Black (1980) and Sumner (1972), respectively. Additionally, chromosomes were stained with Mithramycin (GC-specific) and DAPI (AT-specific) fluorochromes, according to Schweizer (1976).

Repetitive DNA mapping with fluorescence *in situ* hybridization (FISH). FISH (fluorescence *in situ* hybridization) was performed according to Pinkel *et al*. (1986). The 5S rDNA (~200 bp) and 18S rDNA (1400 bp) probes were obtained by polymerase chain reaction (PCR), from the nuclear DNA of *Rachycentron canadum* (Carangiformes),

using the primers A 5′-TAC GCC CGA TCT CGT CCG ATC-3 ′, B 5′-CAG GCT GGT ATG GCC GTA AGC-3 ′ (Pendás *et al*., 1994) and NS1 5′-GTA GTC ATA TGC TTG TCT C-3 ′ / NS8 5 ′ -TCC GCA GGT TCA CCT ACG GA-3 ′ (White *et al*., 1990). The probes were labeled by nick translation with biotin-14-dATP and digoxigenin-11-dUTP (Roche, Mannheim, Germany) and detected with streptavidin-FITC (Vector Laboratories), and anti-digoxigenin-rhodamine (Roche, Mannheim, Germany), respectively.

Microscopy and image processing. At least 30 metaphases of each individual were analyzed and the best results were photographed in an Olympus ™ BX51 epifluorescence microscope coupled to the digital image capture system Olympus DP73 (Olympus Corporation, Ishikawa, Japan), using the cellSens software (Version 1.9 Digital, Tokyo, Kanto, Japan). The fundamental number was based on the number of chromosome arms and the chromosomes were classified as metacentric (m), submetacentric (sm), subtelocentric (st), and acrocentric (a), according to the arms ratio (Levan *et al*., 1964).

Abbreviations. 18S – 18S ribosomal RNA; 2n – Diploid number; 5S – 5S ribosomal RNA; a – Acrocentric chromosome(s); Ag-NORs – Nucleolar Organizing Regions evidenced through silver nitrate impregnation; AT – Adenine/Thymine; DAPI – 4′,6-diamidino-2-phenylindole; FISH – Fluorescence *in situ* hybridization; FITC – Fluorescein isothiocyanate; GC – Guanine/Cytosine; ICMBio – Chico Mendes Institute for Biodiversity Conservation; KCl – Potassium chloride; m – Metacentric chromosome(s); MM – Mithramycin; Mya – Millions of years ago; NF – Fundamental number; NORs – Nucleolar organizing regions; PCR – Polymerase chain reaction; rDNA – Ribosomal DNA; SISBIO – System of Authorization and Information about Biodiversity; SISGEN – National System of Genetic Resource Management and Associated Traditional Knowledge; st – Subtelocentric chromosome(s); µm – micrometer.

RESULTS

Megalops atlanticus has 2n = 50 chromosomes, all acrocentric (NF = 50), while *I. platypterus* has 2n = 48, and the karyotype composed of 2m + 2st + 44a chromosomes (NF = 52) (Fig. 2). No heteromorphic chromosomes were evidenced among the individuals of species.

In both species, heterochromatic blocks occur mainly in the centromeric regions (*e.g*., *M. atlanticus* – pairs 8, 10, 12; *I. platypterus* – pairs 10, 11, 14), but also in the terminal regions of some pairs (*e.g*., *M. atlanticus* – pairs 5, 7, 17; *I. platypterus* – pairs 5, 8, 11) (Fig. 2). The Ag-NORs sites are found in a single chromosome pair, although specific to each species. Thus, in *M. atlanticus* they are interstitially located in the long arms of the smallest 25th pair, while in *I. platypterus* they are terminally located in the short arms of the 2nd pair (Fig. 2, highlighted). These sites are in agreement with the location of the 18S rDNA hybridization signals, being also MM+/DAPI- stained, which characterizes them as GC-rich regions (Fig. 2, highlighted).

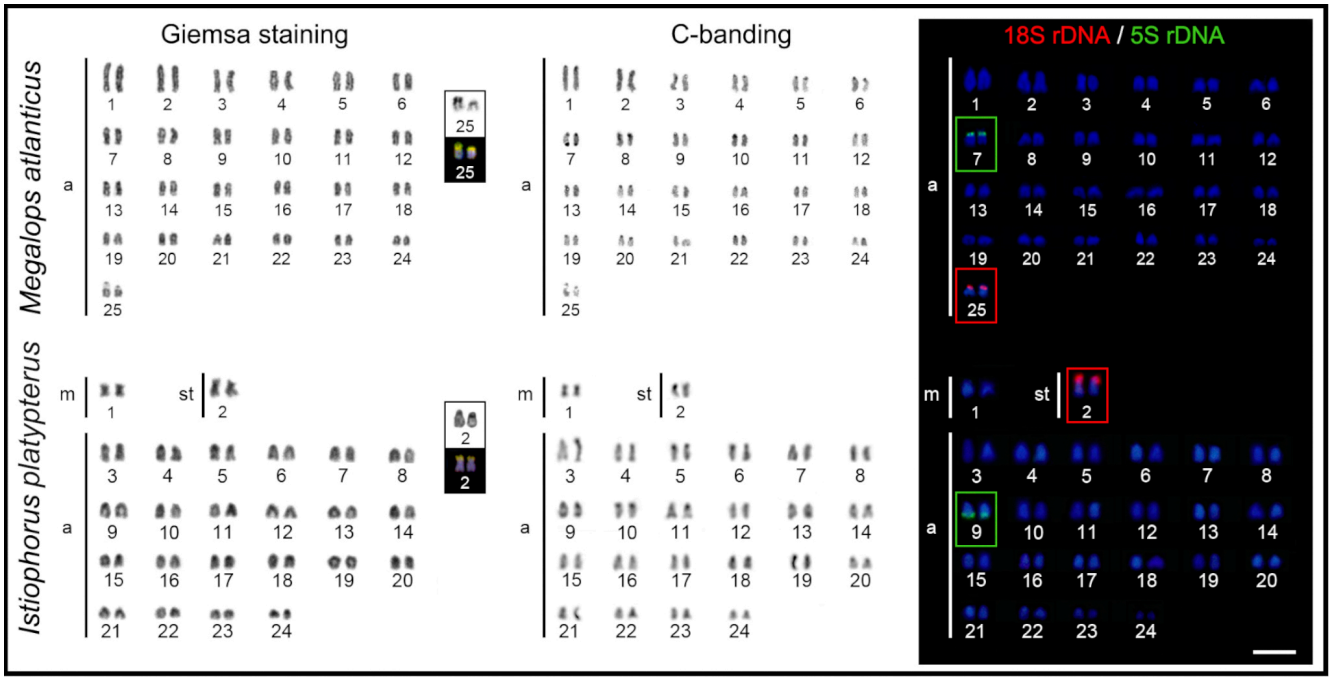

FIGURE 2 | Karyotypes of *Megalops atlanticus* (Megalopidae) and *Istiophorus platypterus* (Istiophoridae) after Giemsa staining, C-banding and FISH procedures. The small left boxes highlight the Ag-NORs and MM+/DAPI- sites, and the right ones the 18S (red) and 5S (green) rDNA sites. Scale bar = 5 µm.

The 5S rDNA sites are located in the short arms of the pair 7, in *M. atlanticus* and in the terminal region of the long arms of the pair 9, in *I. platypterus* (Fig. 2), both acrocentric chromosomes. The $(TTAGGG)_n$ probe hybridized exclusively on the terminal regions of the chromosomes of *M. atlanticus*. In some metaphases of this species, recurrent radial chromosome arrangements were observed (Fig. 2, larger box).

DISCUSSION

Cytogenetic data for large pelagic fishes are sporadic and usually restricted to the description of the diploid chromosome number (Doucette, Fitzsimons, 1988; Khuda-Bukhsh *et al*., 1995; Arai, 2011). This lack of karyotype data for several groups impairs comparative analyzes on their chromosomal relationships and evolutionary trends. In this sense, this study provides classical and molecular cytogenetic data for two representative species, *M. atlanticus* and *I. platypterus.*

Like some other marine pelagic fishes (Accioly *et al*., 2012; Soares *et al*., 2013, 2014), istiophorids with species with large distributions provide a valuable model on karyotype evolution in such ecosystem. However, as commonly found, considerable gaps occur with regard to their cytogenetic characteristics. All cytogenetic information for the Istiophoriformes Order comes down exclusively to the data presented here for *I. platypterus*. Despite this, it is feasible to compare the chromosome patterns of this species with phylogenetically close groups, such as the barracudas (Sphyraenidae),

remoras (Echeneidae), archer fishes (Toxotidae), snooks (Centropomidae), jacks (Carangiformes), flatfishes (Pleuronectiformes), all included in a common clade, the Carangimorphariae one (Betancur-R. *et al*., 2013). It is noteworthy that a large amount of the Carangimorphariae species has 2n = 48 chromosomes (Arai, 2011), but a remarkable diversity in their structural patterns can also be found. In fact, some groups of this clade have exclusively 2n = 48 acrocentric chromosomes, such as Centropomidae (Borges *et al*., 2019) and Toxotidae (Supiwong *et al*., 2017), while other ones like Sphyraenidae (Soares *et al*., 2017), Carangidae (Accioly *et al*., 2012), Echeneidae (Rishi, 1973; Vasiliev, 1980; Arkhipchuk, 1999; Accioly, 2007) and especially Pleuronectiformes (Azevedo *et al*., 2005, 2007), exhibit diversification in the karyotype number and structure.

In a broader phylogenetic context, the karyotype of *I. platypterus* (2m + 2st + 44a; NF = 52) and some features of the repetitive DNA organization in the chromosomes show similarities with species of the Sphyraenidae (Soares *et al*., 2017), and Carangidae (Accioly *et al*., 2012) families, thus supporting a phylogenetic proximity among them. This is true for the independent distribution of the 18S rDNA/Ag-NOR and 5S rDNA sites on chromosomes, a common condition found in different tribes of Carangidae, and also frequent in teleosts (Gornung, 2013). Besides, the terminal location of the 18S rDNA sequences in one of the largest chromosomes of the karyotype is a shared characteristic with several other Carangidae groups (Accioly *et al*., 2012; Jacobina *et al*., 2013), thus suggesting they hold extensive homeologous linkage groups as a plesiomorphic condition.

Megalops atlanticus, with habitats preferably coastal, and *I. platypterus*, which occurs in oceanic regions (Nakamura, 1985), represent model species with high migratory capacity in the marine environment. These species make up groups of low diversity, formed by one genus and two species (Fricke *et al*., 2020) exemplifying the small potential for diversification (Gaither *et al*., 2016), and consequently processes of slow karyotype evolution of large migratory species (RXS, pers. obs.) in the marine environment.

Despite the great dependence on coastal environments, the tolerance to wide variations in salinity and oxygen (Adams *et al*., 2019), migratory habits (Ault *et al*., 2007) and the dispersive potential of larvae (McMillen-Jackson *et al*., 2005), provide favorable conditions for the genetic homogeneity of *M. atlanticus* (McMillen-Jackson *et al*., 2005). It seems that the set of these factors contributes to the karyotype sharing exhibited among populations of the Caribbean (Doucette, Fitzsimons, 1988), with those now presented for the Western Atlantic.

Megalops atlanticus shows microstructural cytogenetic traits also considered as plesiomorphic for several teleosts, such as reduced heterochromatic content, single Ag-NOR/18S rDNA sites (Galetti *et al*., 2000), in non-syntenic arrangement with 5S rDNA sequences (Gornung, 2013). On the other hand, its 2n value (2n = 50) differs from those found for the congeneric species, *Megalops cyprinoides* (Broussonet, 1782), distributed in the Indian and Pacific oceans (Carpenter, Niem, 2001; Nelson *et al*., 2016). In fact, karyotypes with 2n = 46 (Rishi, Haobam, 1984) and 2n = 52 chromosomes (Khuda-Bukhsh *et al*., 1995), were reported for *M. cyprinoides* from two different Indian locations, thus suggesting a more diversified evolutionary condition for this species.

Biogeographically, *M. atlanticus* and *M. cyprinoides* represent two lineages historically isolated by the closing of the Isthmus of Panama – 15–3.1 Mya (Coates, Obando, 1996; Montes *et al*., 2015), separating the Atlantic from the Pacific oceans, and by the Benguela

current – 2 Mya. (Shannon, 1985; Marlow *et al*., 2000), segregating the Atlantic and Indian marine fauna (Henriques *et al*., 2016). However, the opening of the Panama Canal, approximately 100 years ago, provided a new migration route for *M. atlanticus*, from the Caribbean Sea to the Pacific Ocean, and its wide geographical expansion in the Pacific Ocean extending for ~ 2600 km, from Guatemala to the Colombia / Ecuador border (Castellanos-Galindo *et al*., 2019). Given to its migratory potential, the biological invasion of *M. atlanticus* in the Pacific Ocean causes concern for biological conservation. Although no information on sympatry has already been reported, the physical contact could theoretically allow for a genetic introgression between the two *Megalops* species. However, although possible, cytogenetic data demonstrate the occurrence of a heterodiploid condition between them, thus potentiating possible post-zygotic barriers (Yakimowski, Rieseberg, 2014), due to anomalous segregation of their chromosome sets.

Chromosomal diversification also occurs between *Megalops* (Megalopidae) and *Elops* (Elopidae) species, two sister clades of Elopiformes (Tab. 1), in which *Elops saurus* Linnaeus, 1766 shows 2n = 48; 6m/st + 42st/a; NF = 54 (Doucette, Fitzsimons, 1982), while *E. smithi* McBride, Rocha, Ruiz-Carus & Bowen, 2010, has 2n = 50; 6m + 4st + 40a; NF = 60 (Sousa *et al*., 2019). Such differentiations in number and structure suggest that both fusion and fission events have played a role in the karyotype evolution of these Elopiformes families, although apparently associated with other complementary chromosome rearrangements paracentric inversions, translocations, duplications and deletions (Sousa *et al*., 2019). However, the reduced amount of cytogenetic information, coupled with conspicuous karyotypic differences, does not allow for accurate inferences on the evolutionary trends inside this order.

A significant portion of large pelagic marine fish is seriously threatened (Croll, Tershy, 2008) and still lacks on their genetic aspects (Manel *et al*., 2020), including their cytogenetic patterns (Soares *et al*., 2013). In this sense, the present results offer inedit and complimentary cytogenetic data about two important pelagic species, in order to elucidate their karyotype organization. The chromosomal aspects reflect independent evolutionary paths and instigate the extension of the data to other congeneric species and populations, thus providing valuable tools to clarify the evolutionary relationships still largely unknown to Elopiformes.

TABLE 1 | Cytogenetic data for species of Elopiformes Order.

Order	2n	Karyotype	NF	References
Elopiformes				
Elopidae				
Elops saurus	48	6m/st + 42st/a	54	Doucette, Fitzsimons (1982)
Elops smithi	50	6m + 4st + 40a	60	Sousa *et al.* (2019)
Megalopidae				
Megalops atlanticus (Caribbean)	50	50a	50	Doucette, Fitzsimons (1988)
Megalops atlanticus (South Atlantic)	50	50a	50	Present study
Megalops cyprinoides	46	46a	46	Khuda-Bukhsh *et al.* (1995)
Megalops cyprinoides	52	52a	52	Rishi, Haobam (1984)

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ETHICAL STATEMENT

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The authors no declare competing interests.

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