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# Chromosomal evolution in large pelagic oceanic apex predators, the barracudas (Sphyraenidae, Percomorpha)

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Genet. Mol. Res. 16 (2): gmr16029644
Received February 14, 2017
Accepted March 8, 2017
Published April 20, 2017
DOI http://dx.doi.org/10.4238/gmr16029644



**ABSTRACT.** *Sphyraena* (barracudas) represents the only genus of the Sphyraenidae family and includes 27 species distributed into the tropical and subtropical oceanic regions. These pelagic predators can reach large sizes and, thus, attracting significant interest from commercial and sport fishing. Evolutionary data for this fish group, as well its chromosomal patterns, are very incipient. In the present study, the species *Sphyraena guachancho*, *S. barracuda*, and *S. picudilla* were analyzed under conventional (Giemsa staining, C-banding, and Ag-NOR) and molecular ($CMA_3$ banding, and *in situ* hybridization with 18S rDNA, 5S rDNA, and telomeric probes) cytogenetic methods. The karyotypic patterns contrast with the current phylogenetic relationships proposed for this group, showing by themselves to be distinct among closely related species, and similar among less related ones. This indicates homoplasic characteristics, with similar karyotype patterns

originating at least twice, independently. Although still cytogenetically poor investigated, our data were enough to put in evidence a variety of ancient conserved traits and evolutionary novelties for the *Sphyraena* genus. In this sense, it is fundamental that a larger number of Sphyraenidae species, as well as of other phylogenetically related families, be also investigated. This will solidify the knowledge of their karyotypic patterns, and the evolutionary path followed by the species of this particular fish family.

**Key words:** Fish cytogenetics; Sinteny; rDNA; Pelagic fish; Plesiomorphy; Pericentric inversion

## INTRODUCTION

The Sphyraenidae family contains a single genus, *Sphyraena*, whose representatives are popularly known as barracudas. This family consists of 27 species (Eschmeyer and Fong, 2016), including a number of recently identified cryptic ones (e.g., Abdussamad et al., 2015).

Barracudas exhibit a wide range of sizes, with some species growing up to 2 m long. Their bodies are elongated, with silver scales, and strong prominent teeth in the long mandible and roof of the mouth (Russell, 2002). They represent one of the groups of marine apex predators with a high commercial and sport fishing interest (Dunaway, 2008). Their habitats range from the open sea to reefs and estuaries of tropical and subtropical regions (de Sylva, 1963), where they play an important ecological role (de Sylva, 1973). Their great migratory potential is corroborated by phylogeographic analyses, indicating a low or even the absence of any population structure across oceans, overcoming important biogeographic barriers (O'Toole et al., 2011; Daly Engel et al., 2012; Milana et al., 2014).

The evolutionary history of this family indicates an ancient divergence between the living lineages. Although little is known about the various evolutionary aspects of Sphyraenidae, it is estimated that they have originated about 57 mya (Late Paleocene), with the most current lineages belonging to groups that emerged between 23.5-5.3 mya (Miocene) (Santini et al., 2014).

The taxonomic status of Sphyraenidae has drastically changed over time. Recent phylogenetic analyses based on mitochondrial and nuclear sequences suggest the close relationship among Sphyraenidae, Xiphiidae (*swordfish*), and Istiophoridae (*billfish*) families, the last two belonging to the order Istiophoriformes (Betancur et al., 2013). Despite their economic importance (Collette et al., 2011), the evolutionary chromosomal patterns of representatives from these three families, remain unknown (Arai, 2011). Indeed, the little amount of cytogenetic information in pelagic marine species belonging to Sphyraenidae is possibly related to the logistic involved in studying them. To date, there is only one description of the diploid number (2n) and karyotype of a single species from this family, *S. tome*, which displays 2n = 48a chromosomes (Pauls and Coutinho, 1990). Thus, more extensive and detailed analyses will be particularly useful for investigating of karyotype evolution, phylogenetic inferences, and prospection of chromosomal markers from population of intra- and interoceanic regions, as already identified in other families of large pelagic fishes (Soares et al., 2013).

Among the barracudas' species reported for the Western Atlantic, *S. guachancho* (guachanche barracuda), *S. picudilla* (southern sennet), and the circumtropical *S. barracuda* (great barracuda) are relatively common species (de Sylva, 1963) deserving a particular biological

attention. From the taxonomic viewpoint, the validity of *S. picudilla* has been questioned, since it has been suggested a synonymous status of the larvae with *S. tome*, and of the adults with *S. borealis* (Ditty et al., 2006). Similarly, the taxonomic status of *S. tome* is also inaccurate (Ditty et al., 2006). This species is similar to *S. picudilla*, differing by the anal fin count and the number of scales on the lateral line (Menezes and Figueiredo, 1985), but molecular marker data allowing phylogenetic comparisons with another species are still nonexistent.

Thus, in order to contribute to the first cytogenetic survey for the Sphyraenidae family, with the prospection of cytotaxonomic markers and the characterization of its chromosomal evolution, we analyzed the species *S. guachancho*, *S. barracuda*, and *S. picudilla*, all from the Atlantic ocean. The analyses included conventional and molecular cytogenetic methods (Giemsa staining, C-banding, and Ag-NORs detection, base-specific fluorochrome staining and fluorescence *in situ* hybridization (FISH) with 18S rDNA, 5S rDNA, and telomeric probes).

## MATERIAL AND METHODS

### Specimens and obtainment of mitotic chromosomes

Cytogenetic analyses were performed in the species *S. barracuda* Edwards, 1771 (*Great barracuda*; N = 5; 2 females and 3 males) and *S. picudilla* Poey, 1860 (*Southern sennet*, N = 21; 10 females and 11 males), from the Fernando de Noronha archipelago (3°83'47.90"S/32°40'31.46"W), and *S. guachancho* Cuvier, 1829 (*Guachanche barracuda*, N = 8; 4 females and 4 males), from the coast of Rio Grande do Norte (5°88'37.29"S/35°16'65.03"W), in Northeastern Brazil. The collection and handling of specimens followed protocols approved by the Ethics Committee on the Use of Animals of the Federal University of Rio Grande do Norte (Process No. 044/2015).

Mitotic chromosomes were obtained from a short-term culture of cell suspension of kidney tissue fragments (Gold et al., 1990) or a culture of lymphocytes in PbMAX culture medium (Gibco) at 28°C for 72 h (adapted from Moorhead et al., 1960). The cell suspensions were dropped onto slides and stained with 5% Giemsa diluted in phosphate buffer, pH 6.8.

### $CMA_3$, Ag-NOR, and C-banding procedures

The nucleolar organizer regions and the C-positive heterochromatic patterns were analyzed according to the methodologies proposed by Howell and Black (1980) and Sumner (1972), respectively. Moreover, the chromosomes were stained with base-specific fluorochromes chromomycin $A_3$ and 4'-6-diamidino-2-phenylindole (DAPI) (Schweizer, 1980).

FISH with 5S rDNA and 18S rDNA probes was conducted according to Pinkel et al. (1986). The 5S rDNA and 18S rDNA probes were obtained by polymerase chain reaction from the nuclear DNA of *Rachycentron canadum* (Perciformes), using 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). After amplification, the 5S rDNA and 18S rDNA probes were labeled by nick translation with biotin-14-dATP (Invitrogen) and digoxigenin-11dUTP and detected with streptavidin-FITC (Vector Laboratories) and anti-digoxigenin-rhodamine (Roche, Mannheim, Germany), respectively, according to manufacturers' recommendations. The telomeric sequences $(TTAGGG)_n$ were mapped by FISH with the Telomere PNA FISH/FITC kit (DakoCytomation).

## Image processing and data analysis

All analyses were carried out under an Olympus™ BX50 epifluorescence microscope and photographed at a 1000X magnification using the Olympus DP73 digital capture system and the cellSens software (Olympus Optical Co. Ltd.). Around thirty metaphases were analyzed for each specimen to define the diploid number, number of chromosome arms (fundamental number, FN) and prepare representative karyotype for the species. Chromosome types were classified by their arm ratio, as metacentric (m), submetacentric (sm), subtelocentric (st), and acrocentric (a) (Levan et al., 1964). Chromosomal data were analyzed in a phylogenetic context, based on recent molecular phylogeny proposed for Sphyraenidae species (Santini et al., 2014). Although the phylogenetic relationships available do not include *S. tome*, the only other species with a karyotypic description for comparison purposes was tentatively positioned close to *S. picudilla*, given that some of its morphological characters, such as the presence of a pectoral fin that extends past the origin of the pelvic fin, are shared with the latter species (Cervigón et al., 1992; Matsuura and Suzuki, 1997; Smith-Vaniz et al., 1999; Lessa and Nóbrega, 2000).

## RESULTS

All the species have 2n = 48 chromosomes, with variations in the karyotype formula. The karyotypes of *Sphyraena picudilla* and *S. barracuda* are composed of 4sm+4st+40a (FN = 56), while in *S. guachancho* all the chromosomes are acrocentric (FN = 48) (Figure 1). The bi-armed chromosome pairs present in *S. picudilla* and *S. barracuda* have very similar size and morphology (Figure 1). Heteromorphic sex chromosomes or B chromosomes were not found in the analyzed species.

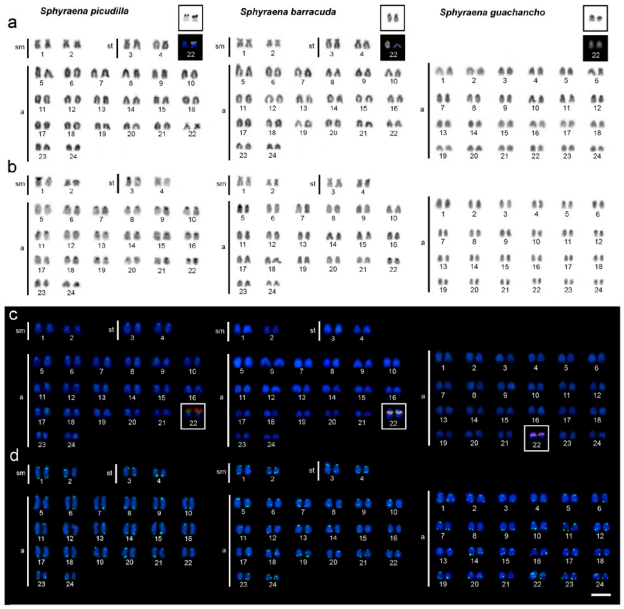


**Figure 1.** Phylogenetic perspective from karyotype patterns and 18S/5S rDNA arrangements in species of *Sphyraena* (adapted from Milana et al., 2014; Santini et al., 2014).

The Ag-NOR ($CMA_3^+/DAPI^-$) sites are unique and located on a small acrocentric pair in all species, identified as pair 22 in the karyotypes. However, in *S. barracuda* and *S. guachancho* they are located in a pericentromeric region on the long arms, while in *S. picudilla* these sites occupy the short arms of this pair (Figure 1a). Small C-positive heterochromatic bands are located in the centromeric and pericentromeric regions of chromosomes being a significant amount coincident with the ribosomal sites (Figure 1b). The 18S and 5S rDNA sites are syntenic and co-localized with the Ag-NOR regions in all the species (Figure 1c). FISH with the telomeric probe shows hybridization signals on each telomere of all chromosomes and interstitial telomeric sites (ITS) were not detected (Figure 1d).

## DISCUSSION

*Sphyraena* species share karyotypes with 2n = 48 and a large number of acrocentric chromosomes. These characteristics are considered basal for Clupeocephala (Brum and Galetti, 1997) and are widely distributed in a number of marine groups (Galetti et al., 2006), including those with significant migratory capacity (Accioly et al., 2012; Soares et al., 2013). Therefore, these karyotype constitution is an ancient characteristic and indicates a conservative symplesiomorphic condition for the family.

The low diversity in the 2n and karyotype macrostructure found in some marine fish groups has been correlated, among other factors, with the maintenance of interpopulation gene flow (Molina, 2007). Indeed, some conditions found in the marine environment, such as few biogeographic barriers, high dispersive potential of the species (both as pelagic larvae or migratory adults), large populations and low ecological requirements, are potential factors for the maintenance of a stable karyotype macrostructure in several fish families (Molina and Galetti, 2004; Soares et al., 2013).

Some barracuda species are able to migrate across vast ocean areas and to maintain high levels of gene flow. In fact, a global phylogeographic survey in *S. barracuda* indicated a high gene flow over long distances in the Indo-Pacific, with genetic divergences in the Western and Eastern Atlantic populations (Daly Engel et al., 2012). Thus, the migratory behavior, besides the extensive period of evolutionary divergence among Sphyraenidae clades and the different historical contexts (multiple transitions between reef and non-reef habitats) that these lineages were submitted (Santini et al., 2014) seem to have mediated their chromosomal evolution. In fact, in addition to the shared features (2n, C-positive heterochromatin distribution, syntenic organization of 18S and 5S rDNA clusters and the large number of acrocentric elements), some few chromosome pairs exhibit divergences derived from pericentric inversions. Indeed, differences in the number of chromosome arms between *S. guachancho* (FN = 48) and *S. barracuda* (FN = 56), species belonging to the same clade, and 18S/5S rDNA arrays clustered in different regions of probable homeologous pairs, support that pericentric inversions that have had a role in the chromosomal diversification of this fish group.

Recent phylogenetic analyses among *Sphyraena* species included *S. barracuda* and *S. guachancho* in the "*Sphyraena barracuda*" clade (Figure 2). The divergence between these species dates from more than 10 mya (Myocene). On the other hand, the "*Sphyraena sphyraena*" clade, which includes *S. picudilla*, has an ancient divergence in comparison with the "*Sphyraena barracuda*" clade, dating from 45 mya (Santini et al., 2014). Based on these data, the karyotypic patterns of the *Sphyraena* species here analyzed have shown to be discrepant with their phylogenetic relationships (Figure 2). In fact, while *S. barracuda* and

*S. picudilla* share similar karyotypes (2n = 4sm+4st+40a), *S. barracuda* and *S. guachancho* exhibit divergent karyotypes (2n = 4sm+4st+40a and 2n = 48a, respectively). The only other Sphyraenidae species with some karyotype information is *S. tome*, displaying 2n = 48a chromosomes (Pauls and Coutinho, 1990). The phylogenetic relationships of this species remain unknown, although it shares a greater morphological similarity with *S. picudilla* than with *S. barracuda* and *S. guachancho* (Matsuura and Suzuki, 1997). In this way, if *S. picudilla* and *S. tome* are indeed more closely related, their karyotypes are less concordant from each other than with species from the other clade (Figure 2). If so, these discrepancies suggest a homoplasic condition in the karyotypic evolution of Sphyraenidae, where same chromosomal patterns were achieved through phylogenetically independent events.

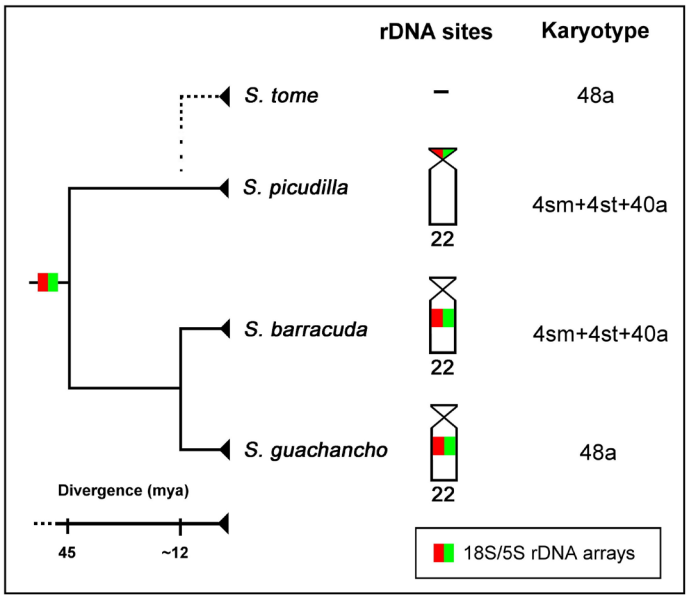


**Figure 2.** Karyotypes of the species *Sphyraena picudilla*, *Sphyraena barracuda*, and *Sphyraena guachancho*. Staining with Giemsa (Ag-NOR and $CMA_3^+$/$DAPI^-$ sites are highlighted) (**a**); C-banding (**b**); double-FISH with 18S rDNA (red) and 5S rDNA (green) probes (**c**); and distribution of the $(TTAGGG)_n$ telomeric sequences (**d**). Scale bar = 5 mm.

In addition, the scarce set of cytogenetic data available for this family did not allow to security infer its basal karyotype. The presence of karyotypes with 2n = 48 acrocentric chromosomes, considered basal for Clupeocephala (Brum and Galetti, 1997), could represent an ancient conserved condition for *S. guachancho* and *S. tome*. On the other hand, the similarity among the bi-armed chromosomes (two sm and two st pairs) of *S. barracuda* and *S. picudilla*, with remote divergence, unlikely occurred by chance. In this sense, it is fundamental that efforts be made to increase the cytogenetic information in a number of Sphyraenidae species, making possible to identify the direction of the chromosomal evolution in this family.

The syntenic 18S/5S rDNA array in the chromosomes of *Sphyraena* deserves a particular consideration. In *S. barracuda* and *S. guachancho*, this gene arrangement occupies the interstitial position of the small acrocentric pair 22, suggesting this preservation during the divergence time of these species. However, in *S. picudilla* (*sphyraena* clade), with the

same chromosome formula of *S. barracuda*, the 18S/5S rDNA array occurs in the terminal position of the same chromosome pair. This divergent location of the rDNA sites between species from different clades, but with similar "basal-like" karyotypes (2n = 48a), strongly suggests that pericentric inversions had occurred involving this chromosome region. Indeed, rDNA regions appear to behave as hotspots for chromosomal rearrangements in fishes. These complex regions, particularly rich in different repetitive sequences (Costa et al., 2015), have been associated with some chromosomal rearrangements, especially with centric fusion events (Getlekha et al., 2016).

$(TTAGGG)_n$ sites are exclusively restricted to the terminal positions of the chromosomes in the three *Sphyraena* species, thus not highlighting chromosomal rearrangements. In fact, the occurrence of interstitial telomeric sites seems particularly prevalent in numerical- structural rearrangements, such as tandem and centric fusions (Getlekha et al., 2016), but rare in pericentric inversion events. However, the absence of interstitial telomeric sites could also be related to the loss of these sequences during the chromosomal rearrangements, the small size of the sequences, or even to its mischaracterization due to accumulated changes in this region (Meyne et al., 1990).

The non-syntenic organization of 45S and 5S ribosomal genes might represent a functional adaptive advantage for them (Martins and Galetti, 2001). The syntenic condition of these genes are less frequent and mainly originated by stochastic evolutionary events limited to one or a few species (Calado et al., 2014), although some fish groups have 18S/5S rDNA syntenic arrangements that are phylogenetically shared (Amorim et al., 2016). However, in *S. picudilla*, *S. barracuda*, and *S. guachancho*, which belong to clades with distant diversification (45 mya), the co-located ribosomal gene organization indicates a symplesiomorphic condition, which appears to be structurally stable and functionally adaptive for this multigene family.

## CONCLUSION

Migratory groups and the absence of specific ecological requirements may contribute to the maintenance of gene flow and consequently lower levels of karyotypic diversification in pelagic marine fishes (Molina, 2007). Large pelagic fishes, such as species of *Sphyraena*, are important models in the investigation of chromosome evolution in migratory marine species. The cytogenetic patterns of *Sphyraena* species are similar to those of other large pelagic migrators, such as some representatives of Scombroidei (Ida et al., 1993; Soares et al., 2013), exhibiting various basal characteristics. By contrast, population groups dependent on particular ecological regions and low vagility may be more susceptible to population stratification and fixation of particular karyotypic patterns (Molina and Galetti, 2004; Molina, 2007). With a complex evolutionary scenario, barracudas had their diversification period in the Oligocene, and since then, have undergone several ecological changes, with alternated preferences for reef and non-reef environments (Santini et al., 2014). Curiously, both *S. guachancho* and *S. tome*, occupying pelagic-neritic habitats (Figueiredo et al., 2002; Santini et al., 2014), and *S. picudilla* and *S. barracuda*, which occupy reef habitats, exhibit similar karyotypes. The cytogenetic data on Sphyraenidae also show the role of pericentric inversions in producing some karyotypic diversification, besides highlighting an uncommon 18S/5S rDNA arrangement that is phylogenetically distributed, evolutionarily stable, and completely functional. However, in view of the limited chromosomal data available for this family, it is essential to obtain additional cytogenetic information for other Sphyraenidae and related

species, making possible to infer about the path of the chromosomal evolution in this peculiar and highly unknown marine group.

## ACKNOWLEDGMENTS

The authors are grateful for the grant provided by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq; #442664/2015-0) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for providing a doctoral fellowship to R.X. Soares. We also thank Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio) by license for collections (#19135-1, #131360-1, and #27027-2) and José Garcia Júnior for help with taxonomic identifications.

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