



## Short communication

# Commercialization of a critically endangered species (largetooth sawfish, *Pristis perotteti*) in fish markets of northern Brazil: Authenticity by DNA analysis



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## ARTICLE INFO

## Article history:

Received 21 January 2013  
Received in revised form  
10 April 2013  
Accepted 11 April 2013

## Keywords:

Largetooth sawfish  
Endangered species  
Molecular identification  
Mitochondrial DNA

## ABSTRACT

*Pristis perotteti*, the largetooth sawfish, is one of most endangered elasmobranchs because of fisheries and habitat degradation. Its commercialization in Brazil is prohibited, but fresh or salted fillets of this fish can be found in markets, labeled as “sharks”. In this study we performed genetic analyses on “shark” samples from two important fishery-trading ports in northern Brazil (Vigia and Bragança). Based on partial DNA sequences of the mitochondrial 16S and Cyt *b* genes, 24 (55%) out of 44 samples were unequivocally identified as *P. perotteti* while the others comprised eight species of the families Carcharhinidae and Ginglymostomatidae. These results show that fishing surveillance and monitoring have not been effective to prohibit the commercialization of this highly endangered species.

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## 1. Introduction

The largetooth sawfish, *Pristis perotteti* Müller & Helle, 1841, is a widespread species in the Atlantic Ocean, inhabiting the American coast and western Africa (Charvet-Almeida et al., 2007; Marko et al., 2004). Currently, the largetooth sawfish ranks as one of the most threatened elasmobranchs due to bycatch in fisheries and degradation of coastal environments (Burgess, Carvalho, & Imhoff, 2009). Therefore, *P. perotteti* is listed in the IUCN as a critically endangered (CR) A2abcd (Charvet-Almeida et al., 2007).

Fishing and trade of *Pristis* species have been illegal in Brazil since 2004. Nonetheless, sawfish are caught as bycatch in fisheries targeting commercially important species in northern Brazil such as swordfish (*Xiphias gladius*), common dolphinfish (*Coryphaena hippurus*), tuna (*Thunnus* spp.) and Piramutaba catfish (*Brachyplatystoma vaillant*).

Since the monitoring system is ineffective, the meat of *Pristis* species is found fish markets along the northern Brazilian coast under common unspecified trade names. Fresh or salted fillets are

usually found in local markets along coastal regions without any label but the at generic “shark” (“cação”) tag, inasmuch as identification at species level based on meat appearance is an impossible task. “Cação” is a popular name used in Brazil to refer to the meat of several elasmobranch species, particularly sharks.

The proper identification of fish products, like fillets, is a worldwide concern and several studies have focused on unreported or misidentified fishing issues. In these cases, DNA analyses represent a useful tool for determining the authenticity of fishery resources (Woolfe & Primrose, 2004).

Using molecular approaches, several authors revealed that high-value fish species are often replaced by low-value ones, leading to economic losses (Armani, Castigliero, Tinacci, Gianfaldoni, & Guidi, 2011; Cawthorn, Steinman, & Witthuhn, 2011; Jacquet & Pauly, 2008; Von der Heyden, Barendse, Seebregts, & Matthee, 2010) and misinterpretation of fish stocks (Crego-Prieto, Campo, Perez, & Garcia-Vazquez, 2010; Marko et al., 2004). In the case of elasmobranchs, analyses of DNA sequences have been useful to detect fraudulent misidentification of shark (Barbuto et al., 2010) and ray species (Lago, Vieites, & Espiñeira, 2012; Marko et al., 2004).

In this study, the partial sequencing of two mitochondrial genes (16S and Cyt *b*) was used to identify fresh and salted fillets of “cação” (sharks) in order to check if *Pristis* species are found in

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northern Brazil markets. This method is important to track down elasmobranch-derived products (meat and fins) commercialized in the region or exported to other countries and eventually identify endangered species such as sawfish.

## 2. Material and methods

### 2.1. Sampling, amplification and sequencing

A total of 44 samples of fresh and salted fillets, labeled as “cação” (sharks), available in fish markets of Bragança (Bra) and Vigia (Vig), state of Pará, northern Brazil, were analyzed. Both markets are major trading ports of marine fish in Brazilian northern coast (PROZEE, 2006) and the local people suggested that these samples might include largemouth sawfish.

The salted tissue samples were washed, at least five times, in bidistilled water. Total DNA was extracted from all samples using a phenol-chloroform procedure, followed by precipitation in sodium acetate and isopropanol (Sambrook & Russell, 2001). The gene fragments (Cyt *b* and 16S) were amplified via polymerase chain reaction (PCR), using the following primers pairs: 16S rRNA: 16S L 1987 5'-GCCTCGCTGTTTACCAAAAAC-3' and 16S H 2609 5'-CCGGTCTGAACCTCAGATCAG-3' (Palumbi et al., 1991); Cyt *b*: PriscBF1 5'-TGAGGACAAATATCCTTCTGAGGTGC-3' and PriscBR1 5'-AAGTAGGTGATTGAGGCCGATTTGGCC-3' (Feldheim et al., 2010).

Each PCR reaction comprised 4 µl of dNTP solution (1.25 mM), 5 µl of buffer (10X), 2 µl of MgCl<sub>2</sub> (50 mM), 0.25 µl of each primer (200 ng/µl), 0.2 µl of *Taq* polymerase (5 U/µl), 1 µl of template DNA (200 ng/µl) and bidistilled water to a final volume of 25 µl. The amplification program encompassed an initial step at 94 °C for 3 min, 35 cycles at 94 °C for 30 s, 1 min at specific annealing temperature (16S rRNA – 50 °C; Cyt *b* – 56 °C), and 72 °C for 2 min and a final extension step at 72 °C for 7 min.

Sequencing was carried out using a Big Dye kit (ABI Prism™ Dye Terminator Cycle Sequencing Reading Reaction – PE Applied Biosystems). The reaction was performed in a final volume of 10 µl, using 2.0 µl of each primer (0.8 pmol/µL), 1 µl of PCR product, 1 µl of Big Dye mix, 1.5 µl of buffer and 4.5 µl of distilled water and run on

an ABI Prism 3500 automatic sequencer (Applied Biosystems). All newly generated sequences were submitted to GenBank under accession codes XXX-XXX.

### 2.2. Analyses of molecular data

All sequences were edited and aligned using the software Bio-Edit 5.0.6 (Hall, 1999). To identify species of each sample, the DNA sequences were compared to a genetic database of elasmobranchs from the Brazilian coast, available in the Genetics and Molecular Biology Laboratory at Coastal Studies Institute (IECOS), and also blasted against the GenBank database (Table 1). One sample of *P. perotteti* and other six ray species from Amazon coast genetically identified in a previous report (Carmona, Sampaio, Santos, Souza, & Schneider, 2008) were kindly donated by CEPNOR and used as comparison in the present analyses (Table 1). The identification criterion was established on a similarity level of at least 99%, i.e., a P (not corrected) nucleotide divergence lower than 1%, combined with a neighbor-joining phylogenetic analysis based on P distance, both available in MEGA 5.0 (Tamura et al., 2011).

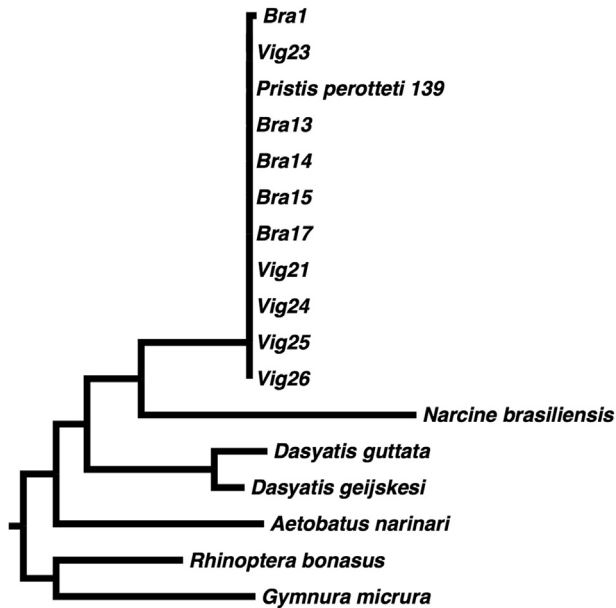
## 3. Results and discussion

The initial screening with 16S sequence (425 bp) showed that 23 out of the 44 (55%) samples were 100% identical to *P. perotteti*, while one sample (Bra1) had a single divergent nucleotide compared to *P. perotteti* (Fig. 1). We found the other 20 samples to be highly similar to shark species of two families common in northern Brazil, Carcharhinidae and Ginglymostomatidae (Fig. 2).

Amongst the 20 shark samples, 18 were unambiguously identified to the species level: three *Carcharhinus leucas* specimens (Bra47, Bra57 and Vig27), two *Carcharhinus limbatus* (Bra42 and Bra43), four *Carcharhinus porosus* (Bra46, Bra51, Bra53 and Vig 22), three *Carcharhinus acronotus* (Bra16, Bra45 and Vig40), one *Sphyrna lewini* (Bra50), two *Galeocerdo curvier* (Bra48 and Vig28) and three *Ginglymostoma cirratum* specimens (Bra35, Bra39 and Bra49). Only two samples (Bra37 and Vig51) belonged to one Carcharhinidae-like species (0.2% of divergence between both samples) that could

**Table 1**  
Samples included in the present work.

Family	Scientific name	Common name	GenBank code		Reference
			16S	Cyt <i>b</i>	
Carcharhinidae	<i>Carcharhinus leucas</i>	Bull Shark	XX-XX		Present study
	<i>Carcharhinus limbatus</i>	Blacktip Shark	XX-XX		Present study
	<i>Carcharhinus porosus</i>	Smalltail Shark	XX-XX		Present study
	<i>Carcharhinus acronotus</i>	Blacknose Shark	AY147897		Present study
	<i>Sphyrna lewini</i>	Scalloped Hammhead Shark	HM239662		Unpublished
	<i>Galeocerdo cuvier</i>	Tiger Shark	HM239661		Unpublished
Ginglymostomatidae	<i>Ginglymostoma cirratum</i>	Nurse Shark	AY147890		(Douady, Dosay, Shivji, & Stanhope, 2003)
Pristidae	<i>Pristis perotteti</i>	Largetooth Sawfish		D50024	(Kitamura, Takemura, Watabe, Taniuchi, & Shimizu, 1996)
	<i>Pristis perotteti</i>	Largetooth Sawfish	XX-XX	XX-XX	Present study
	<i>Pristis microdon</i>	Freshwater Sawfish		GU139180	(Feldheim et al., 2010)
	<i>Pristis clavata</i>	Dwarf Sawfish		GU139179	(Feldheim et al., 2010)
	<i>Pristis zijsron</i>	Narrow snout Sawfish		GU139185	(Feldheim et al., 2010)
	<i>Pristis pectinata</i>	Smalltooth Sawfish		GU139175–78	(Feldheim et al., 2010)
	<i>Anoxypristis cuspidata</i>	Knifetooth Sawfish		GU139181	(Feldheim et al., 2010)
	Dasyatidae	<i>Dasyatis guttata</i>	Longnose Stingray	XX-XX	
<i>Dasyatis geijskesi</i>		Sharpsnout Stingray	XX-XX		Present study
Myliobatidae	<i>Aetobatus narinari</i>	Spotted eagle ray	XX-XX		Present study
Narcinidae	<i>Narcine brasiliensis</i>	Brazilian electric ray	XX-XX		Present study
Gymnuridae	<i>Gymnura micrura</i>	Smooth butterfly ray	XX-XX		Present study
Rhinopteridae	<i>Rhinoptera bonasus</i>	Cownose ray	XX-XX		Present study

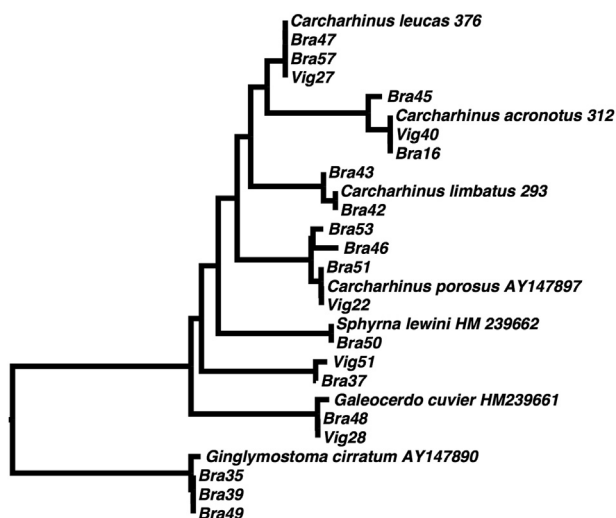


**Fig. 1.** Neighbor-joining tree based on nucleotide P (not corrected) divergences of a 425 base pairs alignment of the 16S mitochondrial gene of comparing the unknowing fish fillets against ray species. Only 10 of 24 samples are showed. The remaining 14 samples are 100% identical to *P. perotteti* 139.

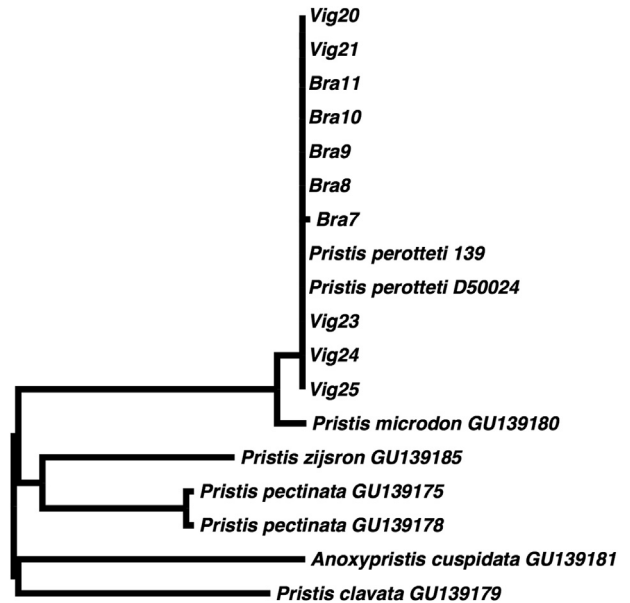
not be identified to the species level as similar sequences were neither found in GenBank nor in our database (Fig. 2).

Sequencing of Cyt *b* (493 bp) was performed in 10 out of the 24 samples identified as *P. perotteti* by 16S analysis, five from Bragança and five from Vigia. The samples were confirmed to be *P. perotteti*; nine of them were 100% identical to the control sample (*P. perotteti* 139) and to the *P. perotteti* from GenBank D50024 (Feldheim et al., 2010). One sample, Bra7, differed by one nucleotide compared to the *P. perotteti* reference samples (Fig. 3). All ray samples used in our comparisons, either from GenBank of 16S sequences or from our database of Brazilian species (Cyt *b*), were clearly differentiated by nucleotide divergence.

This study shows that species identification based on DNA sequencing of short genome fragments is useful for the discrimination of fish products when morphological differentiation is no



**Fig. 2.** Neighbor-joining tree based on 16S mitochondrial gene comparing unknowing fish fillets against shark species.



**Fig. 3.** Neighbor-joining tree based on nucleotide P (not corrected) divergences of a 493 base pairs alignment of the Cyt *b* mitochondrial gene of comparing the unknowing fish fillets against ray species.

longer viable. Moreover, this approach corroborated other studies by showing that misidentification of commercial fish is a common practice in fisheries trade.

For instance, using Cyt *b* sequences, Marko et al. (2004) showed that 70% of red snapper (*Lutjanus campechanus*) from U.S. markets were actually less valuable species of Lutjanidae. Another report, based on 16S sequences, showed high levels of misidentification in fish fillets in the South Africa trade (Von der Heyden et al., 2010).

In the case of elasmobranchs, two reports are noteworthy. Firstly, Barbutto et al. (2010) detected 77.8% of frauds in filets labeled as “palombo” (genus *Mustelus*) in Italian markets by using DNA sequence of the mitochondrial COI gene. Recently, Lago et al. (2012) tested the efficiency of COI sequence in identifying 20 ray filets, named as *Raja* spp. in Spain, and found out that only four samples belonged to *Raja* (20%), while 16 (80%) represented the genera *Amblyraja*, *Bathyraja* and *Leucoraja*.

The present analysis and the above mentioned reports reveal how serious this situation is both at economic (frauds) and environmental (stock management) viewpoints. Furthermore, the utility of DNA markers for species identification in fisheries is confirmed, validating them as valuable tools for the authenticity and investigation of fish products.

## Acknowledgment

This work has been funded by CNPq and FAPESPA.

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