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DNA barcoding for the identification of swim bladders: An approach to international trade monitoring

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ABSTRACT

The rapidly expanding global trade in fish maw (dried swim bladders) necessitates urgent forensic monitoring, as this highly processed commodity is morphologically unidentifiable, directly facilitating the illicit inclusion of vulnerable species in the legal supply chain. This research applied DNA Barcoding (cytochrome oxidase I; COI gene) to 120 fish maw samples seized by Brazilian environmental authorities at a major international airport to provide species-level identification and crucial data on trade practices. We identified eight species, with the overwhelming majority belonging to Amazonian Sciaenids (*Cynoscion acoupa* and *Plagioscion auratus)* and the catfish *Sciades parkeri*. Crucially, the definitive identification of *C. acoupa* and *S. parkeri* confirms the direct exploitation of species listed as Vulnerable by the IUCN, tracing their flow from multiple Brazilian states to major global consumer hubs, including Hong Kong, the United States, United Kingdom and China. Furthermore, the study exposed a critical methodological constraint: an initial ambiguous species assignment by the BOLD system (94 % match) was only resolved by confirming the correct species, *S. parkeri*, through the NCBI database (100 % match). This finding demonstrates the essential need for critical evaluation and the utilization of complementary reference libraries to overcome 'code gaps' in forensic analyses. These data underscore the essential role of molecular techniques as an enforcement tool for traceability and provide unequivocal evidence supporting the immediate need for targeted legislation and rigorous regulatory oversight to protect vulnerable Amazonian stocks.

1. Introduction

Marine fisheries have historically served as a vital source of food, economic activity, and cultural identity across civilizations [1]. However, globally, they are increasingly linked to unsustainable practices that pose a threat to marine biodiversity [2]. Technological advancements since the Industrial Revolution have enhanced fishing efficiency and productivity but have also intensified the depletion of fishery resources [3]. In Brazil, the fishery and aquaculture industries have expanded significantly, as evidenced by a rise in exports [4]. Nonetheless, overexploitation of fish stocks raises serious concerns about the sustainability of the sector. The absence of accurate data on national fishery activities impairs proper resource management [5], while predatory fishing practices further threaten marine ecosystems [6].

In this context, the commercialization of specific marine animal parts, such as swim bladders and fins, has gained substantial value in international markets particularly in China, Japan, the UK, the US, and Germany [1]. The swim bladder is a hydrostatic organ found in fish, composed of multiple layers, including an outer fibrous collagen-rich membrane [7]. Isinglass, a swim bladder derivative, is widely utilized in various industries, from food and beverages to traditional medicine, notably for clarifying beer and wine [1,7,8]. The trade in fish swim bladders, locally known as 'grude' or internationally as fish maw, constitutes a high-value global commodity chain [9,10]. The annual import volume and value of this product are comparable to other established seafood delicacies, such as shark fins and sea cucumber [9]. This immense demand, particularly from markets in China and Hong Kong [10], has led to a significant escalation in fishing pressure across various

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source countries. This situation is particularly critical because the most commercially valued species, such as croakers (Sciaenidae), often form spawning aggregations, which makes them exceptionally susceptible to overexploitation [10].

Collagen extracted from swim bladders via acetic acid processing has diverse applications in film, pharmaceuticals, and biomedical fields [7, 8]. However, these products are typically traded in uncharacterized forms, hindering species identification. The increasing interest in fish maw drives the expansion of fisheries into new regions and for new species [9], thereby intensifying the risk of overexploitation of resources that are poorly managed or unprotected [10]. According to Diálogo Chino [11], global imports of swim bladders between 2015 and 2020 reached 20,000 tons, valued at HK$14 billion (US$1.8 billion), with Brazil as the leading exporter (3,300 tons; HK$3 billion or US$385 million). This trade is driven by the demand for fish maw collagen, particularly from species endemic to northern Brazil.

As a leading global exporter, Brazil finds itself at the core of this conservation challenge, underscoring the necessity of forensic and monitoring tools, for effective supply chain oversight. Therefore, molecular techniques have become indispensable for verifying the species involved [12]. Modern molecular taxonomy, introduced by Tautz et al. and Hebert et al. [13,14], relies on DNA as the primary means of species identification. Mitochondrial DNA analysis is especially useful in fish species authentication and fishery management [15]. DNA barcoding, as proposed by Hebert et al. [14,16], enables the identification of species through short, standardized mitochondrial DNA sequences of the gene COI. This method is highly effective for recognizing both known and novel species. The Barcode of Life Data System (BOLD) [17] serves as a global database that facilitates sequence matching and enforces quality standards for reliable species assignment [18]. Mitochondrial DNA's high mutation rate further enhances its utility in forensic and taxonomic identification.

Given the significant economic importance and escalating commercial exploitation of the fish swim bladder byproduct (known globally as 'fish maw' or 'isinglass') amidst a critical lack of official trade data, this study aims to apply molecular markers to identify swim bladders seized by environmental inspection authorities at the export sector of Guarulhos International Airport. The primary goal is to provide crucial data regarding the species involved in this commerce and enhance international trade monitoring efforts.

2. Materials and methods

The samples were collected during inspection actions conducted by the Brazilian Institute of Environment and Renewable Natural Resources (IBAMA) in the São Paulo-Guarulhos International Airport "Governador André Franco Montoro" during 2022 (Fig. 1). A total of 120 swimming bladders obtained from completely dried material from morphologically uncharacterized fish.

Small pieces of tissue of each individual were submitted to DNA extraction using the PureLink™ Genomic DNA Mini Kit (ThermoFisher, USA) following the manufacturer's protocol with adjustments to the Proteinase K digestion (2 h). Due to the processed and dehydrated nature of the material, agarose gel electrophoresis was not feasible. DNA integrity was assessed using the Qubit™ dsDNA BR Assay Kit (Invitrogen, USA) and the Qubit® 2.0 Fluorometer. Fragments of approximately 650 base pairs (bp) of the COI gene were amplified by polymerase chain reaction (PCR) using the pair of primers COI Fish1 Foward (5'-ACGCCTGTTTATCAAAAACAT-3') and COI Fish2 Reverse (5'-ACTTCAGGGTGACCGAAGAATCAGAA-3') [19]. PCR reactions were conducted in a thermocycler - ProFlex PCR System (Applied Biosystems) at a total volume of 14 µl, including 1 µl of genomic DNA (10–30 ng/µl); 1.25 µl of 1 × PCR buffer (20 mM Tris-HCl, pH 8.4, and 50 mM KCl); 0.38 µl of 1.5 mM $MgCl_2$; 150 µM of each dNTP in a final volume of 1.5 µl; 0.2 µl of *Taq* polymerase (Invitrogen, Brazil); 0.75 µl with a concentration of 10 nmol/µl of each primer and 8.2 µl of Milli-Q H_2O. The cycling conditions were an initial denaturation of 95ºC for 5 min followed by 35 cycles at 95°C for 30 s; 52°C for 30 s; 72°C for 45 s and a final extension of 72°C for 5 min. Before sequencing, PCR products were purified using the enzyme kit "Exo-SAP IT" (USB Corporation, Brazil). PCR products were visualized in 2 % agarose gel stained with GelRed® Nucleic Acid Stain 10000X (Sigma, Brazil) and observed in transilluminator under UV light. PCR sequencing reaction was done using the "Big Dye Terminator v.3.1 Cycle Sequencing Ready Reaction" (Applied Biosystems, Brazil) kit using the same primers used before. The solution for each reaction contained 10–30 ng/µl of genomic DNA, 1X BigDye

Fig. 1. Sampling of dried swim bladders seized at the export control center of Guarulhos International Airport. A – Sampling of a swim bladder fragment for storage. B – Variation in size and shape of collected swim bladders. C – Boxes intended for international shipment containing dried swim bladders.
Source: Prepared by the author (2024).

buffer, 5X Bigdye and 0.1– 0.4 µl of primer, with a total volume of 7 µl. The cycling conditions were an initial denaturation at 96ºC for 2 min followed by 35 cycles at 96°C for 30 s; 50°C for 15 s and 60°C for 4 min.

Samples were sequenced using an ABI 3500 Genetic Analyzer (Applied Biosystems), and the data were subsequently curated in Geneious 7.1.3. The presence of stop codons was also verified within the Geneious software to ensure the translational quality of the COI gene fragments. For the initial species assignment, our sequences were queried against the BOLD Systems database [17] and National Center for Biotechnology Information (NCBI) [19] using BLAST searches.

Sequences exhibiting a minimum identity of 98 % to reference barcodes were retained. The top hits from these searches were retrieved and combined with our dataset, generating a final alignment comprising 132 sequences (70 newly generated sequences plus 62 reference sequences retrieved from the databases). Multiple sequence alignment was carried out using the Geneious alignment algorithm implemented in Geneious v7.1.3. The final alignment dataset was used to construct a Neighbor-Joining (NJ) tree in MEGA v12.0.14 under the Kimura 2-parameter (K2P) model, allowing visualization of clustering patterns and validation of the taxonomic assignment. The resulting tree was subsequently edited and visualized in FigTree v1.4.4 (https://github.com/rambaut/figtree).

3. Results and discussion

The collected materials were dehydrated and morphologically unidentifiable. These items were shipped internationally via postal services, often disguised among food products (e.g., instant noodles), electronics, or within passenger luggage (Fig. 2).

Although labeled as swim bladders a legally tradable item the absence of documentation prompted their seizure. Of the 120 samples analyzed, eight fish species were identified: 50 *Cynoscion acoupa*, 11 *C. leiarchus,* 3 *C. microlepidotus*, 17 *C. virescens*, 2 *Macrodon ancylodon*, 1 *M. atricauda*, 34 *Plagioscion auratus* and 2 *Sciades parkeri*. Among the identified species, *C. acoupa* and *S. parkeri* are categorized as "Vulnerable" by the IUCN Red List, while the remainder are categorized as "Least Concern" (Table 1).

Considerable intra-specific variation was observed among some sequences in the phylogenetic analysis (Fig. 3 and Fig. 6 - see supplementary material -). Due to the highly degraded nature of the DNA in the analyzed materials - resulting from the way these products are processed and traded - the overall sequencing quality was suboptimal, which may have contributed to this observed variation. However, similar levels of divergence were also identified among reference sequences already deposited in the BOLD Systems database, suggesting that this pattern likely reflects not only technical limitations but also genuine natural genetic variability within these species.

Table 1
Summary of identified species, detailing the common name, conservation status according to the IUCN Red List, and the quantity recorded.

Species	Common Name	IUCN Red List Status	Quantity
Cynoscion acoupa	Acoupa Weakfish	Vulnerable	50
Cynoscion leiarchus	Smooth Weakfish	Least Concern	11
Cynoscion microlepidotus	Smallscale Weakfish	Least Concern	3
Cynoscion virescens	Green Weakfish	Least Concern	17
Macrodon ancylodon	King Weakfish	Least Concern	2
Macrodon atricauda	Southern King Weakfish	Least Concern	1
Plagioscion auratus	Black curbinata	Least Concern	34
Sciades parkeri	Gillbacker Sea Catfish	Vulnerable	2

The utility of DNA Barcoding in the forensic identification, though undeniably powerful [20], is inherently limited by its reliance on comprehensive public reference databases, where the absence of high-quality sequences creates so-called 'code gaps' [18]. This technical fragility is critically exposed by the highly dynamic global fish maw trade [9], as overwhelming international demand drives the rapid and often unregulated expansion of exploitation into new species at a pace that the deposition of their genetic profiles frequently fails to match [10].

This study provides empirical evidence of this limitation: while two sequences (15049 and 15058, see supplementary material) were initially assigned to *Sciades couma* by the BOLD Systems [17] with a compatibility score of only 94 %, submission of the identical sequences to the NCBI [19] resulted in a definitive identification of *Sciades parkeri*, achieving 100 % compatibility. This notable discrepancy demonstrates that the quality and coverage profiles of reference libraries across different databases directly influence species assignment and can lead to questionable results when reliance is placed solely on low compatibility scores [18,20]. This challenge is further aggravated by the highly processed and morphologically unidentifiable state of the seized samples [9], which eliminates any possibility of traditional taxonomic verification, thereby underscoring a significant enforcement challenge imposed by the processing practices of the illegal trade [10].

Fig. 2. Materials in boxes and suitcases intended for international shipment, seized by agents of the IBAMA at the export control center of Guarulhos International Airport. A – Storage of the seized material in a room at the export control center by agents of the IBAMA. B – Swim bladder being shipped together with instant noodles. C – Swim bladders for consumption being shipped separately in plastic bags.
Source: Prepared by the author (2024).

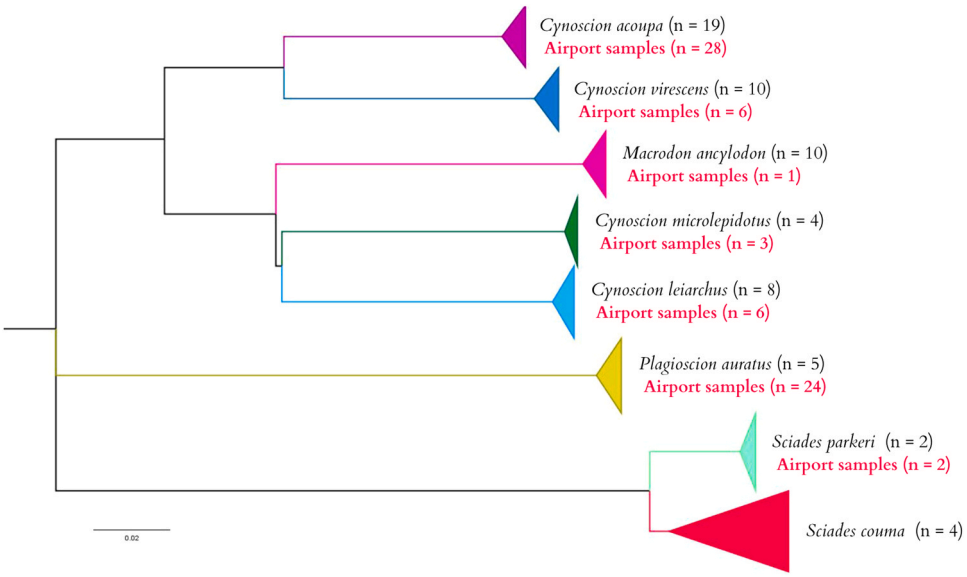

Fig. 3. Simplified Neighbor-Joining (NJ) phylogenetic tree based on COI gene sequences. In italic typeface the respective sample codes obtained from public databases (BOLD/NCBI) and in red the sequences obtained from the seized samples at Guarulhos International Airport. 'n' represents the total number of sequences clustered in that branch.
Source: Prepared by the author (2025).

The seized products originated from multiple Brazilian locations: Recife (PE), São Paulo (SP), Belém (PA), São Caetano do Sul (SP), and Volta Redonda (RJ) and were destined for Hong Kong, the United States, the United Kingdom, and China (Fig. 4). Only the species for which both dispatch and destination information was available were included in the flow diagram. Samples seized from passenger luggage, rather than mail shipments—where such information can be retrieved—lacked dispatch and destination records and were therefore not considered in this step. Consequently, the flow diagram displays six of the eight identified species.

Our findings, which illustrate a flow of Brazilian fish swim bladders destined for Hong Kong, the United States, the United Kingdom, and China, are consistent with documented global trade patterns for fish maw [9,11]. Hong Kong, specifically, is recognized as a primary global center for the import and re-export of this product [9,11]. The prevalence of Sciaenids, including the identified *Cynoscion* and *Macrodon* species, along with the catfish (*Sciades parkeri*), reflects the dominance of these families in international trade, as detailed in global analyses [9].

The high proportion of endemic Amazonian species identified, such as *Cynoscion acoupa* (42 %) and *Plagioscion auratus* (28 %), highlights the international market's dependency on resources from Northern Brazil.

In 2020 alone, Brazil exported 637 tons of swim bladders, a 398 % increase from the 127 tons exported in 2012 [11]. Most exports originated from Pará, aligning with the species identified in this study (Fig. 5), suggesting that these fish were harvested in this region.

According to dos Santos & de Oliveira [21], the fish maw trade in Vigia (Pará) operates under a capitalist dynamic, concentrating profits among a few large-scale traders and providing minimal benefit to artisanal fishers and riverside communities. This reflects weak public policies regulating fish maw commercialization in Brazil.

The confirmed presence of vulnerable species in this trade underscores the urgent need for robust regulatory measures. The history of the fish maw market offers severe precedents: high-value demand has driven species like the Chinese Bahaba (*Bahaba taipingensis*) and the Totoaba (*Totoaba macdonaldi*) to the brink of critical extinction [9,10]. This risk is directly tied to the product's exorbitant value, which

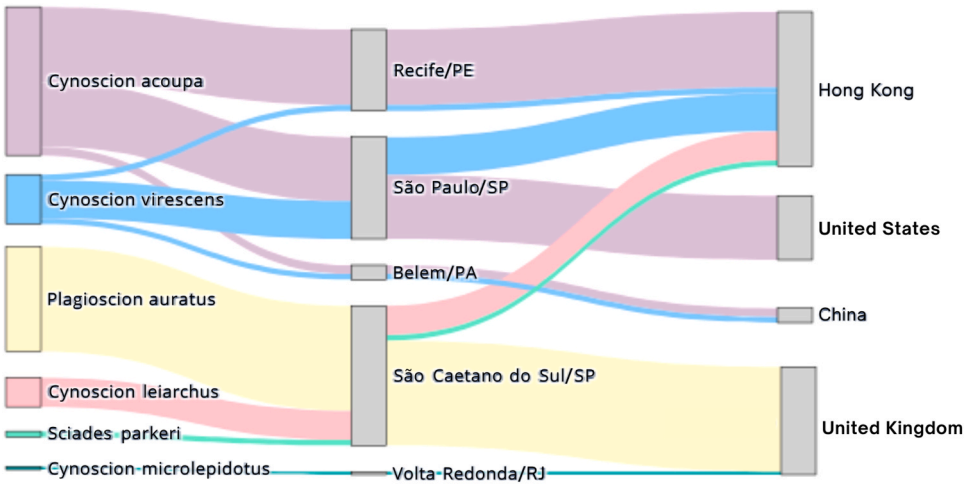

Fig. 4. Flow diagram representing the collected samples, the cities of origin in Brazil, and the international destination cities.
Source: Prepared by the author (2025).

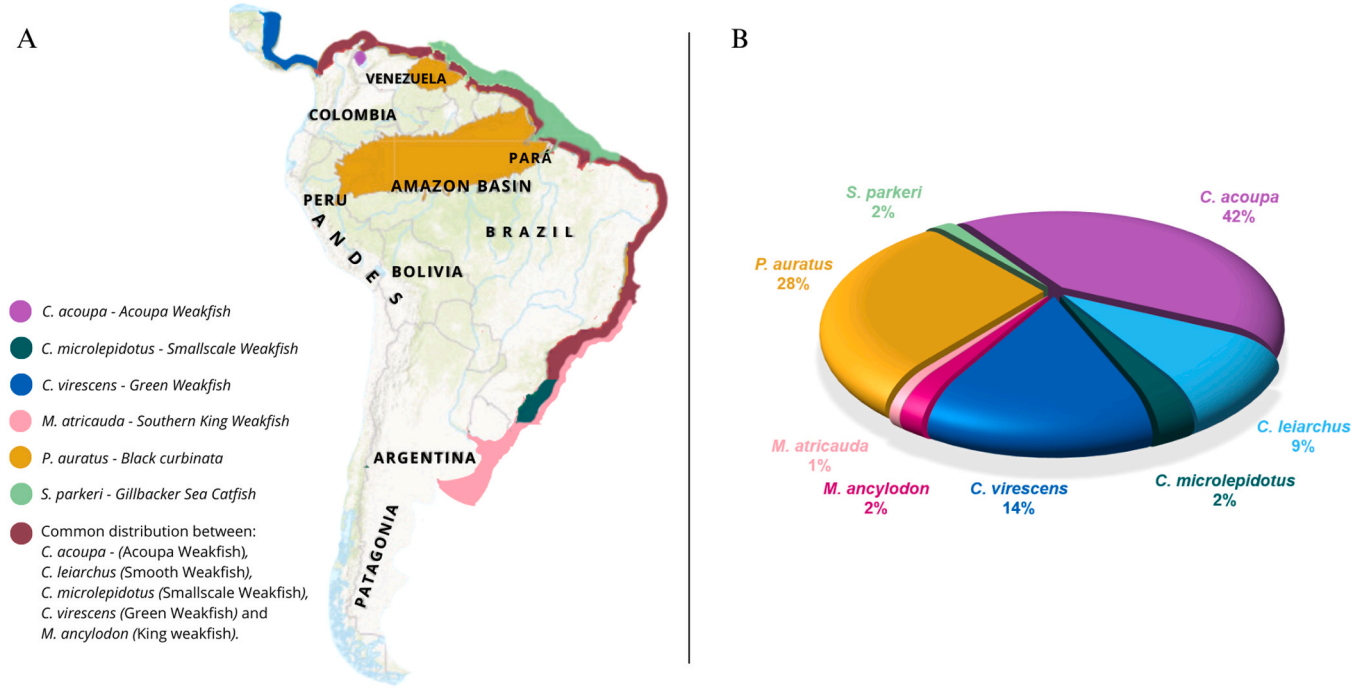

Fig. 5. A - The spatial distribution map of the species identified in this study is presented. B - A pie chart illustrates the relative abundance proportions of each species.
Source: Modified from IUCN [22].

incentivizes fishing efforts that overwhelm local management capacity [10]. A similar scenario may be unfolding with *C. acoupa* and *S. parkeri*, both currently listed as "Vulnerable" by the IUCN, with *C. acoupa* also categorized as Near Threatened on Brazil's National Red List [22].

Our application of DNA Barcoding to identify the vulnerable species *C. acoupa* and *S. parkeri* confirms the tool's essential role as a forensic method for mitigating the risk of overexploitation. These data collectively reinforce the global call for more stringent legislation and targeted enforcement [9], particularly in Brazil, where regulatory ambiguity and a lack of supply chain traceability have been documented.

4. Conclusion

This study firmly underscores the value of molecular forensic tools in the authentication and traceability of traded wildlife products. Specifically, DNA Barcoding provides environmental enforcement agencies with the essential means to conduct species-level identification where the processed, dried, and often morphologically unidentifiable nature of the samples fails conventional methods. Our data, demonstrating a trade highly dependent on species from the Amazon Basin, supports the urgent need for targeted legislation, rigorous enforcement, and the development of economic incentives to ensure compliance and minimize ecological impacts on species listed as Vulnerable by the IUCN, such as *Cynoscion acoupa* and *Sciades parkeri*. Furthermore, we suggest the inclusion of the species identified in this study under the CITES framework to enhance their protection, given that the trade in swim bladders continues to expand annually, posing an increasing threat to the species involved.

CRediT authorship contribution statement

Freitas Gabriela Idaline Idaline: Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Daniel Eduardo Visciano de Carvalho:** Writing – original draft, Methodology, Funding acquisition. **Rodrigues Jr Carlos Egberto:** Writing – original draft, Methodology, Funding acquisition, Formal analysis. **Fabio Portofori:** Writing – original draft, Supervision, Project administration, Methodology, Funding acquisition, Formal analysis. **Ricardo Utsunomia:** Writing – original draft, Methodology, Investigation.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.fsir.2025.100440.

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