

Identification of *Squalus* spp. in the Argentine-Uruguayan common fishing area (ZCPAU) using genetic DNA barcoding

Abstract

The *Squalus* genus is divided into three taxonomic categories that comprise various species, although their nomenclature is frequently inconsistent across different ichthyologists and taxonomies. In the Atlantic Ocean, there are three known species of spiny sharks, *S. acanthias*, *S. cubensis*, and *S. mitsukurii*, with discrepant classifications. During campaigns conducted by the INIDEP Chondrichthyan Fisheries Program, 27 *Squalus* specimens from the Argentine-Uruguayan Common Fishing Zone (ZCPAU) and Argentine coast were collected and morphologically identified onboard. We then coupled DNA COI Barcode technology with BLASTn and BOLD IDS tools to molecularly validate those taxonomic classifications. *COI* gene sequences were generated for those 27 specimens and compared to available reference sequences (FISHBOL database) from *S. acanthias*, *S. cubensis*, *S. mitsukurii*, *S. suckleyi*, *S. blainville*, and *S. megalops* from different oceanic regions. Genetic distances (K2P) and phylogenetic relationships (via neighbor-joining phylogenetic tree and Median-joining haplotype network) were also estimated for the *COI* data. A total of thirteen samples identified by the BOLD Identification System did not match the *Squalus* species identified using morphological evidence. Our analyses also indicated that *S. mitsukurii*, *S. acanthias*, and *S. cubensis* are three clearly distinct species, while *S. lobularis* is deemed equivalent to *S. mitsukurii* (K2P=0.35%). Our haplotype network also confirmed the existence of *S. acanthias*, *S. cubensis*, and *S. mitsukurii* in the studied area. The application of these molecular techniques and analyses have proved effective at accurately identifying *Squalus* species; hence we recommend their use to validate former morphology-based taxonomic identifications.

Keywords: squalidae, sharks, COI, south western atlantic, barcode

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Introduction

Squalidae is considered one of the most challenging shark groups to classify;¹ it consists of two genera: *Cirrhigaleus*, which contains three species, and *Squalus*, which taxonomic composition is still debated due to significant challenges in species differentiation caused by its considerable morphological similarity.² *Squalus* is usually subdivided in three distinct groups, although their taxonomic names can vary among experts. Bigelow and Schroeder³ were pioneers in describing these groups using morphological evidence; they studied specimens from the western Atlantic Ocean and categorized them into *acanthias*, *blainville-fernandinus*, and *megalops* or *brevirostris-cubensis*. Later, these researchers renamed these categories after their most ancient representative, resulting in *S. acanthias*, *S. fernandinus*, and *S. megalops*.⁴ Garrick⁵ proposed a revised naming system based on similar traits but with certain changes, identifying *S. fernandinus* (Molina, 1782) as a synonym for *S. acanthias*, thereby creating the groups: *S. acanthias*, *S. blainville*, and *S. megalops - cubensis*. Cadenat and Blache⁶ also identified three species groups, providing an identification key for the *S. acanthias*, *S. blainville*, and *S. megalops* groups. Compagno et al.⁷ and later researchers^{8,9} also recognized them as *S. acanthias*, *S. mitsukurii*, and *S. megalops*, which is the nomenclature currently in use. Nonetheless, the identification of these *Squalus* groups is still unclear and its taxonomy remains controversial. In the southwestern region of the Atlantic continental shelf situated between 34°S and 41°S, two spiny shark species from the *Squalus* genus were recorded: *S. acanthias* and *S. blainville*.¹⁰ The sightings of *S. blainville* along the shores of Buenos Aires and Uruguay are likely to correspond to *S. cubensis*, while those recorded in deeper waters

of the shelf are probably of *S. mitsukurii*.¹¹ Some researchers have even questioned the existence of *S. cubensis* in the region, suggesting that these sightings might actually be *S. megalops*.¹² In a subsequent taxonomic review of chondrichthyans found in the southwestern Atlantic Ocean below 34°S, Menni and Lucifora¹³ indicated that there are three species of spiny dogfish: *S. acanthias*, *S. cubensis*, and *S. mitsukurii* (Figure 1).

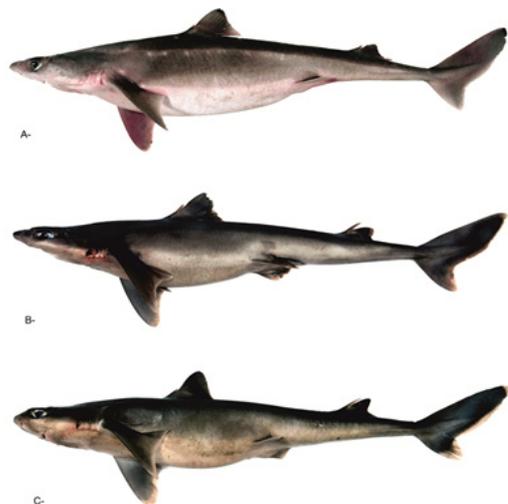


Figure 1 *Squalus* species present in the Southwest Atlantic, a- *S. acanthias*, b- *S. mitsukurii* and c- *S. cubensis*.¹⁶

The species *S. cubensis* is classified within the *S. megalops* group, whereas *S. mitsukurii* is linked to the *S. mitsukurii* group.⁸ Current taxonomic key guides developed to identify sharks of the genus *Squalus* from this area^{14,15} effectively assist in recognizing *S. acanthias* (skin with noticeable white spots, the spine of the first dorsal fin originates just behind the inner margin of the pectoral fins), but they pose challenges at distinguishing between *S. mitsukurii* and *S. cubensis* (both with smooth, spotless skin, the spine of the first dorsal fin originates just above the inner margin of the pectoral fins). Key features available for identification include the diagonal distance from the snout tip's center to the inner edge of the nostrils, which is longer in *S. mitsukurii* and shorter in *S. cubensis*, as well as the type of dermal denticles, tricuspidate in *S. mitsukurii* and unicuspidate in *S. cubensis*. However, these characteristics are difficult to evaluate during research expeditions or commercial trips.¹⁶ Consequently, utilizing molecular methods such as DNA barcoding of the mitochondrial gene for the enzyme cytochrome C oxidase subunit 1 (COI) can aid significantly in differentiating between *Squalus* species. The goal of this study was then to use DNA COI barcoding to molecularly identify *Squalus* specimens collected from the Argentine-Uruguayan Common Fishing Zone and the Argentine coast morphologically identified onboard as *S. acanthias*, *S. cubensis*, *S. lobularis* and *S. mitsukurii*. By doing this, we expect to resolve ongoing issues related to their taxonomic classification. Additionally, we will compare them to other *Squalus* species from different oceans and latitudes to determine their patterns of geographic distribution in their area of occurrence.

Materials and methods

The Department of Molecular Genetics and Microbiology at INIDEP received muscle samples from 20 *Squalus* specimens from 5 fishing hauls of the vessel BIP Eduardo Holmberg during the EH03/13 campaign, as well as 7 samples caught during a single haul of the same ship during the EH02/16 research effort. The locations where these specimens were collected are within the Argentine platform and the Argentine-Uruguayan Common Fishing Zone (ZCPAU) (Figure 2). Onboard the vessel, the specimens were identified to the species level using morphological features.

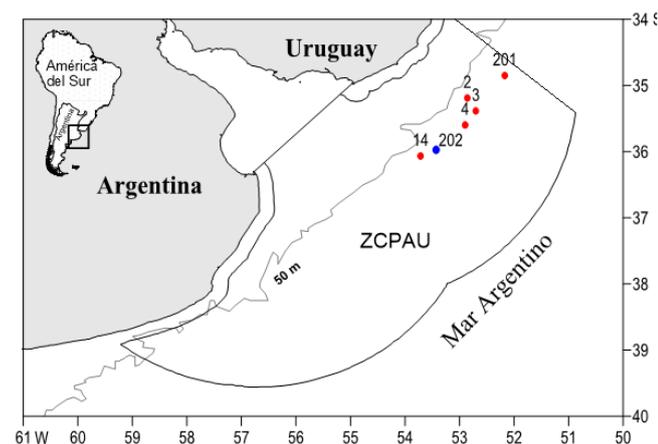


Figure 2 Location of the fishing hauls where the *Squalus* specimens under study were caught. Hauls from the EH03/13 campaign (20 specimens) and from the EH02/16 campaign (7 specimens) are indicated in red and blue, respectively.

DNA extraction and PCR amplification

DNA was isolated from 50-100 mg of muscle tissue using phenol-chloroform-isoamyl alcohol; EB buffer (200mM Tris pH 7.5,

250mM 5M NaCl, 25mM EDTA pH 8.0, 0.5% SDS) was used instead of CTAB in the DNA extraction, following Andreoli and Trucco.¹⁷ About 600 base pairs of the *COI* gene were amplified by PCR with the universal primers FishF2 (5' TCG ACT AAT CAT AAA GAT ATC GGC AC 3') and FishR1 (5' TAG ACT TCT GGG TGG CCA AAG AAT CA 3') as outlined by Ward et al.¹⁸ Every PCR reaction was performed in a total volume of 25 μ l, which included: 1X PCR buffer (1.5 mM MgCl₂), dNTPs (0.2 mM), primers (0.5 μ M), 0.625 U of Taq polymerase (GoTaq DNA polymerase from Promega), and 2 μ l of the DNA template. The amplification was conducted using a BioerLife Express TC-96/G/H thermal cycler with the following settings: an initial denaturation step of 2 min at 95° C; followed by 34 cycles consisting of 1 min at 94° C, 1 min at 54° C, and 2 min at 72° C; and a final extension of 15 min at 72° C. A negative control was used to ensure lack of contamination in all reactions. The integrity of the amplified products was evaluated through 1.5% agarose gel electrophoresis, with visualization accomplished using 0.01% ethidium bromide. The purified amplification products were then sequenced in both directions utilizing the Sanger technique at the Genomics Unit of INTA-Castelar, Argentina (<http://www.inta.gov.ar>).

Sequences analyses

Each sequence underwent manual review to identify uncalled or miscalled bases and all variable positions were verified by cross-referencing the sequence reads generated from both the forward and reverse strands of each individual. Manual editing of chromatograms and the assembly of both strands were carried out with the BIOEDIT v.5.0.6 program.¹⁹ The generated sequences were submitted to Genbank and assigned accession numbers OR707029 to OR707032 for *S. acanthias*, OR707039 to OR707043 for *S. cubensis*, and OR707044 to OR707055 for *S. mitsukurii*. The identification of each sequence was done through the Barcode of Life Data System (BOLD,²⁰ using the Species Level Barcode Records option. Our *COI* sequences were combined with other *COI* reference sequences of *S. acanthias*, *S. cubensis*, *S. mitsukurii*, *S. blainville*, *S. megalops*, and *S. suckleyi* from the FISHBOL database, resulting in a final data set of 65 reference sequences. These species came from various locations, including the South and North Pacific, the Southwestern Atlantic, New Zealand, Italy, and the Gulf of Mexico (see Appendix). *COI* sequences were aligned using the Clustal W method and a model of evolution estimated. Phylogenetic relationships and genetic distances were inferred using the Neighbor-Joining (NJ) method under the best-fit model of evolution (K2P). Tree uncertainty was assessed using bootstrap analysis (5,000 replicates). An external outgroup sequence from the mandarin dogfish of the Squalidae family, *Cirrhigaleus barbifer* (Tanaka, 1912) (Genbank accession number 398719.1), was used to root the tree. Since our main goal was to identify *Squalus* species using *COI* barcodes and the BOLD methods, we did not consider necessary to use other more powerful maximum likelihood and Bayesian approaches of phylogenetic inference. All these analyses were performed in MEGA 6.²¹ The DnaSP v.5.1 package²² was also used to estimate haplotypes, variable sites, and haplotype diversity. Network haplotype relationships with their geographical distributions were inferred using the Median-Joining method²³ in Network 10.1.²⁴

Results

Genetic identification of specimens obtained on board

The *COI* gene served as a useful molecular marker for recognizing specimens from the three *Squalus* species. The BOLD Identification System enabled species-level identification, achieving similarity percentages >99% for the best matches in every instance. Each

specimen evaluated corresponded to the species *S. acanthias*, *S. cubensis*, or *S. mitsukurii*, which can be found in the area (Table 1). A total of thirteen samples identified by the BOLD Identification System did not match the *Squalus* species identified using morphological evidence. Five samples morphologically identified as *S. cubensis* were barcoded as *S. acanthias* (Squs 9, 13, and 16) and *S. mitsukurii* (Squs 5 and 6). Similarly, one sample morphologically identified as *S. acanthias* (Squs 11) was actually *S. mitsukurii*, and seven samples labeled as *S. lobularis* were confirmed to be *S. mitsukurii* (Table 1). These misclassifications involving *S. acanthias*, *S. cubensis*, and *S. mitsukurii* highlight the difficulty and ambiguity in using morphological features to distinguish these *Squalus* species effectively.

Table 1 Identification of *Squalus* specimens using the BOLD system

Sample Name	Morphol. Identific.	BOLD best hit	Reference sequence accession number	Identity percentage
Squs 1	S. mitsukurii	S. mitsukurii	GNSHK097-08 Squalus mitsukurii	99,85
Squs 2	S. cubensis	S. cubensis	GBGC9594-09 Squalus cubensis FN431670.1	99,69
Squs 3	S. mitsukurii	S. mitsukurii	FARG335-07 Squalus mitsukurii EU074610	100
Squs 4	S. mitsukurii	S. mitsukurii	FARG335-07 Squalus mitsukurii EU074610	100
Squs 5	S. cubensis	S. mitsukurii	FARG335-07 Squalus mitsukurii EU074610	99,85
Squs 6	S. cubensis	S. mitsukurii	FARG333-07 Squalus mitsukurii EU074611	100
Squs 7	S. mitsukurii	S. mitsukurii	FARG335-07 Squalus mitsukurii EU074610	100
Squs 9	S. cubensis	S. acanthias	FARG228-06 Squalus acanthias COI-5P EU074604	100
Squs 10	S. acanthias	S. acanthias	FARG228-06 Squalus acanthias COI-5P EU074604	100
Squs 11	S. acanthias	S. mitsukurii	GNSHK096-08 Squalus mitsukurii	100
Squs 12	S. acanthias	S. acanthias	FARG228-06 Squalus acanthias COI-5P EU074604	100
Squs 13	S. cubensis	S. acanthias	FARG228-06 Squalus acanthias COI-5P EU074604	100
Squs 14	S. mitsukurii	S. mitsukurii	FARG335-07 Squalus mitsukurii EU074610	100
Squs 15	S. cubensis	S. cubensis	GBGC9594-09 Squalus cubensis FN431670.1	99,85
Squs 16	S. cubensis	S. acanthias	GNSHK129-08 Squalus acanthias	99,85
Squs 17	S. cubensis	S. cubensis	GBGC9594-09 Squalus cubensis FN431670.1	100
Squs 18	S. cubensis	S. cubensis	GBGC9594-09 Squalus cubensis FN431670.1	100
Squs 19	S. cubensis	S. cubensis	GNSHK047-11 Squalus cubensis	99,84
Squs 20	S. cubensis	S. cubensis	GBGC9594-09 Squalus cubensis FN431670.1	99,85
Squs 21	S. mitsukurii	S. mitsukurii	FARG335-07 Squalus mitsukurii EU074610	100
S.lob0216L202-1	S. lobularis	S. mitsukurii	FARG335-07 Squalus mitsukurii EU074610	100
S.lob0216L202-2	S. lobularis	S. mitsukurii	FARG335-07 Squalus mitsukurii EU074610	100
S.lob0216L202-3	S. lobularis	S. mitsukurii	FARG335-07 Squalus mitsukurii EU074610	100
S.lob0216L202-4	S. lobularis	S. mitsukurii	FARG335-07 Squalus mitsukurii EU074610	100
S.lob0216L202-5	S. lobularis	S. mitsukurii	FARG333-07 Squalus mitsukurii EU074611	100
S.lob0216L202-6	S. lobularis	S. mitsukurii	FARG335-07 Squalus mitsukurii EU074610	100
S.lob0216L202-7	S. lobularis	S. mitsukurii	GNSHK100-08 Squalus mitsukurii COI-5P	99,69

Phylogenetic relationships among *Squalus* species

Evolutionary relationships among selected *Squalus* samples were inferred using the NJ approach under the K2P model of evolution (Figure 3). The same model was used to estimate distances between *Squalus* species (Table 2). The NJ tree (Figure 3) depicted three distinct clades supported by bootstrap proportions (bp) of $\geq 99\%$. GROUP 1

contained sequences of *S. acanthias* and *S. suckleyi* grouped in two subclades (bp = 100%) and showed a K2P distance of 0.83%. The second clade (GROUP 2) encompassed sequences of *S. blainville*, *S. megalops*, and *S. cubensis*. Within this cluster, the sequences numbered 19, 2, 15, 18, 17, and 20 were placed alongside *S. cubensis*, affirming their classification. Furthermore, the tree depicted a clear distinction between *S. cubensis* and *S. blainville* or *S. megalops* (bp = 100%), also supported by K2P distances >1.50 ; this confirms *S. cubensis* is a different species from the other two. The third clade (GROUP 3) comprised *S. mitsukurii* and the specimens identified as *S. lobularis* (*S. lob#* in the tree). Both the NJ topology and the distance metrics indicated that the putative *S. lobularis* and *S. mitsukurii* are probably members of the same species. The distance between them was 0.35%, which overlaps with the intraspecific distances estimated for the other species (*S. mitsukurii* K2P=0.37 and *S. lobularis* K2P=0.33, data not shown).

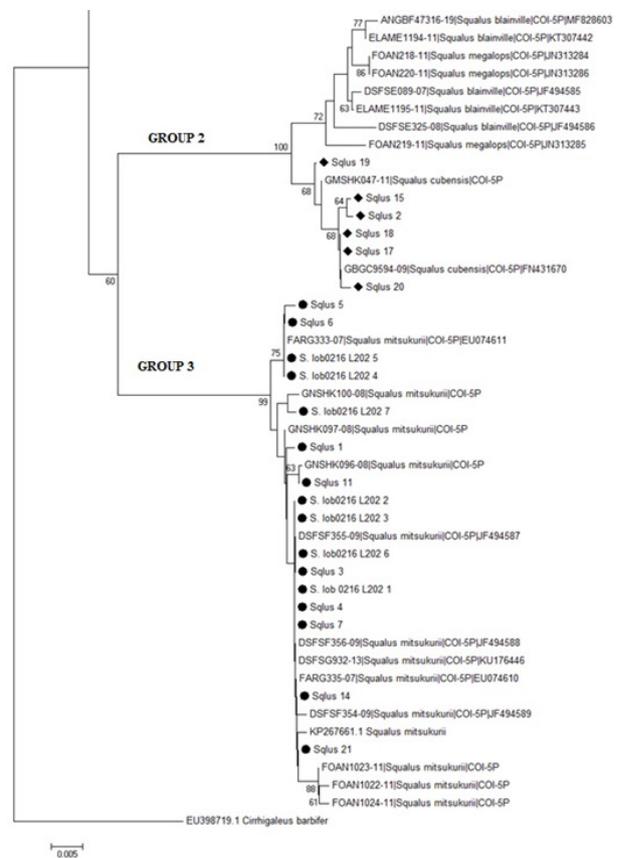


Figure 3 Neighbor joining tree of COI sequences from the *Squalus* species analyzed in this work (indicated with different symbols for each group) and those obtained from the FISHBOL database. Bootstrap proportions $>50\%$ are indicated.

Table 2 K2P distances between species expressed as percentages

	<i>S. acanthias</i>	<i>S. suckleyi</i>	<i>S. blainville</i>	<i>S. megalops</i>	<i>S. cubensis</i>	<i>S. lobularis</i>	<i>S. mitsukurii</i>
<i>S. acanthias</i>							
<i>S. suckleyi</i>	0.83						
<i>S. blainville</i>	8.08	7.90					
<i>S. megalops</i>	8.18	7.68	0.82				
<i>S. cubensis</i>	7.78	7.30	1.73	1.53			
<i>S. lobularis</i>	7.05	6.28	6.74	6.89	6.30		
<i>S. mitsukurii</i>	7.19	6.38	6.79	6.97	6.25	0.35	

To study the relationships between the different *Squalus* species and their geographical distribution, a Median-Joining haplotype network was estimated (Figure 4). From the analysis of 92 sequences with 691 sites, 47 haplotypes were obtained, with a total of 86 variable sites, and a haplotype diversity (Hd) of 0.9247. Three distinct groups of *Squalus* were again obtained, GROUP 1 (for the acanthias-suckleyi group), GROUP 2 (for the megalops-blainville-cubensis group) and GROUP 3 (for mitsukurii-lobularis). Within each group the species showed a wide distribution, as evidenced by the diversity of colours in the haplotypes. The presence of the red colour, which corresponds to the South Atlantic, showed that it is possible to find species from all three groups (acanthias, cubensis and mitsukurii) in the ZCPAU and Mar Argentino. This analysis also confirmed that *S. acanthias* and *S. suckleyi* are distinct species. Although the number of mutational steps between the two species is low (three), a marked differentiation by distribution was observed. The haplotypes of the *S. suckleyi* sequences are not shared with those of *S. acanthias* and were limited to the North Pacific (the only haplotypes in black), while the haplotypes of *S. acanthias* had a wider distribution, from the South Atlantic (red) to the North Atlantic (green), South Pacific (grey), Italy (blue) and New Zealand (yellow). For group B, the species *S. blainville* and *S. megalops* were limited to Italy (blue) and South Africa (violet) and no shared haplotype was observed (i.e., two different species). *S. cubensis* occurred between the North (green) and South Atlantic Ocean (red), confirming its presence in our region and its separation from the other *Squalus* species, as also suggested by the NJ tree. Concerning *S. mitsukurii* and *S. lobularis*, our network also showed that they are actually the same species, not differentiated by their geographical locations, as they share identical haplotypes (in the predominant H1, sequences from both kinds are present). The sequences originating from Taiwan and southern China (orange haplotypes) are likely to be included in this group. The outlined distribution pattern is presented in Table 3.

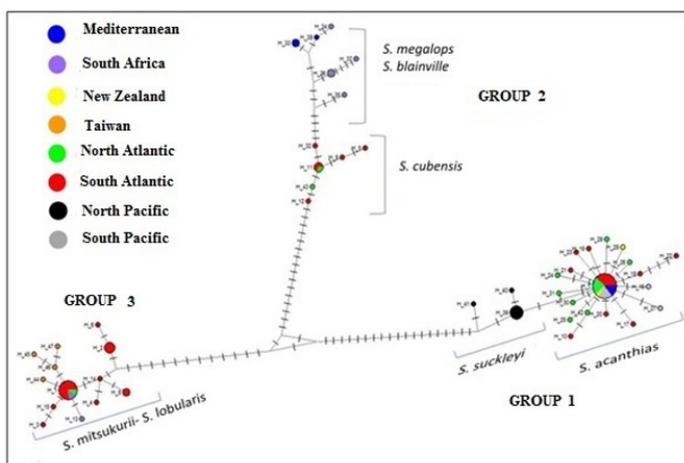


Figure 4 Median-joining haplotype network. The size of the circles is proportional to the haplotype frequency.

Table 3 Distribution pattern of the *Squalus* species analyzed in this study

<i>Squalus</i> species	Distribution
<i>S. acanthias</i>	North Atlantic Ocean
	South Atlantic Ocean
	Mediterranean
	New Zealand
<i>S. suckleyi</i>	South Pacific
	North Pacific

Table 3 Continued...

<i>S. megalops</i> y <i>S. blainville</i>	Mediterranean
	South Africa
<i>S. cubensis</i>	North Atlantic
	South Atlantic
	North Atlantic
<i>S. mitsukurii</i> - <i>S. lobularis</i>	South Atlantic
	South Africa
	Taiwan

Discussion

This study shows that DNA barcoding of *COI* sequences can be applied to identify and classify the occurrence of *Squalus* species in the southwestern Atlantic. This molecular approach is more accurate than using morphological evidence since external physical traits (the shape of dorsal, pectoral, and anal fins, color patterns and the dimensions of the dermal denticles) often overlap among various species leading to *Squalus* misidentification. The variation in classification between *S. lobularis* and *S. mitsukurii* underscores the complicated taxonomic status of these species, stemming from the categorization suggested by Viana et al.²⁵ during their comprehensive global review of *Squalus*. These researchers conducted a regional reassessment of specimens from Brazil, Uruguay, and Argentina to determine the valid species in the southwest Atlantic Ocean. By carrying out an in-depth comparative study of the external features and skeletal structures of *Squalus* specimens in the region, they proposed that *S. mitsukurii* and *S. cubensis* were absent. Our findings contradict that conclusion since the genetic distance estimated between the specimens identified morphologically as *S. lobularis* and *S. mitsukurii* suggested that they actually are the same species (K2P= 0.35%). This was further validated by the observation of that both species shared haplotypes and occupied the same geographic regions, indicating that *S. mitsukurii* is present in the southwestern Atlantic. It is crucial to note that the research conducted by Viana et al.²⁵ lacked molecular analysis and relied exclusively on morphological features.

Ariza et al.²⁶ adopted the nomenclature of *S. albicaudus* for the Atlantic specimens from the Brazilian coast, following Viana et al.²⁵ Their study covered specimens from the southwestern Atlantic and the Pacific. The K2P distance found by these authors between *S. albicaudus* and *S. cubensis* was 0.0072, so they speculated that *S. albicaudus* might encompass a population of *S. cubensis* in the Southeastern Atlantic Ocean that is presently experiencing speciation – such process would be relatively new and not entirely finished, given that these species continue to exhibit shared haplotypes. The distribution to the southern region of Brazil is unknown.²⁵ The DNA Barcode method applied in this study also allowed us to distinguish between *S. acanthias* and *S. suckleyi*, which corroborated previous findings by Ebert et al.⁹ regarding the differentiation between these two species using morphological, meristic and molecular data. The distance between both species analyzed in this study was 3 to 7 times greater than their respective intraspecific distances, which also agreed well with the distances in Ebert et al., who reported an interspecific distance up to 5 or 6 times greater. *S. acanthias* can be easily differentiated from the other two species found in the area due to the presence of white spots on its sides; however, distinguishing between *S. mitsukurii* and *S. cubensis* is more complicated. The main differences between these two species lie in the length of the preanial region and the specific type of dermal denticles.^{14,15} These features are challenging to identify during research expeditions because *Squalus* sharks in the area often gather in groups, with some sets yielding up to 900 kg of *S. mitsukurii*. This high volume per set complicates the

detailed analysis of individual specimens.¹⁶ This may explain why two male specimens (Squalus 5 and 6) were initially identified as *S. cubensis* aboard and later identified through BOLD as *S. mitsukurii* (100% similarity). Additionally, six out of the eleven specimens labeled as *S. cubensis* showed 100% or nearly identical similarity to *S. cubensis* sequences from the Gulf of Mexico, which contrasts with the findings of Viana et al.²⁵ regarding this species' limited distribution in the southwestern Atlantic. Accurate identification of species is crucial for implementing conservation strategies and ensuring the responsible use of natural resources. The species studied in this work are considered by the International Union for Conservation of Nature (IUCN) as vulnerable (*S. acanthias*), endangered (*S. mitsukurii*)²⁷ and least concern (*S. cubensis*).²⁸ In 2006, the "Spiny Shark" item was added to the official fishing statistics of Argentina. It appears as Dogfish sharks nei, so landings reported under this category may include any of the three species considered here.²⁹ As Colonello et al.¹⁶ observed, it is important to enhance the identification of observable traits that can be quickly and easily recognized in *S. mitsukurii* and *S. cubensis* samples. This will facilitate research on the biology, habitat preferences, and stock dynamics of populations and species of *Squalus* in the area.

Conclusion

The current research established that *S. cubensis* and *S. mitsukurii* can be accurately identified through DNA COI barcode analysis. It also showed that the two species form distinct evolutionary lineages, as indicated by our NJ phylogeny, K2P distances and Median Joining haplotype network. Moreover, the network analysis confirmed the presence of *S. mitsukurii*, *S. acanthias*, and *S. cubensis* in the Argentine Sea and the Argentine-Uruguayan common fishing area (ZCPAU). Therefore, since these species cannot be easily distinguished aboard vessels, we recommend the use of the molecular methods employed in this study to validate initial morphological assessments and reach accurate taxonomic identifications.

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Conflicts of interest

The authors declared that there are no conflicts of interest.

References

- Ebert D, Winton M. *Chondrichthyans of high latitude seas*. In: Carrier J, Musick J, Heithaus M, editors. The biology of sharks and their relatives. CRC Press LLC; 2010.
- Last P, White W, Pogonoski J, et al. Application of a rapid taxonomic approach to the genus *Squalus*. In: *Descriptions of new dogfishes of the genus Squalus (Squaloidea: Squalidae)*. Last PR, White WT, Pogonoski JJ, editors. CSIRO; 2007. pp. 117–130.
- Bigelow HB, Schroeder WC. *Sharks*. In: Tee Van NJ, Breder CM, Hildebrand SF, et al, editors. *Fishes of the Western North Atlantic*. Part 1. Mem Sears Found Mar Res, Yale University, New Haven; 1948. pp. 1–576.
- Bigelow HB, Schroeder WC. A study of the sharks of the suborder *Squaloidea*. *Bull Mus Comp Zool Harvard Univ*. 1957;117(1):1–150.
- Garrick JAF. *Studies on New Zealand Elasmobranchii*. Part XII. The species of *Squalus* from New Zealand and Australia; and a general account and key to the New Zealand Squaloidea. *Trans R Soc New Zealand*. 1960;88(3):519–557.
- Cadenat J, Blache J. Requirements of the Mediterranean and the Atlantic (more particularly the West African Coast). *Tropical Fauna ORSTOM*; 1981:330.
- Compagno L, Dando M, Fowler S. *Sharks of the world*. Princeton University Press; 2005.
- Ward R, Holmes B, Zemlak T, et al. DNA barcoding discriminates spurdogs of the genus *Squalus*. In: *Descriptions of new dogfishes of the genus Squalus (Squaloidea: Squalidae)*. Last PR, White WT, Pogonoski JJ, editors. CSIRO; 2007:117–130.
- Ebert DA, White WT, Goldman KJ, et al. Resurrection and redescription of *Squalus suckleyi* (Girard, 1854) from the North Pacific, with comments on the *Squalus acanthias* subgroup (Squaliformes: Squalidae). *Zootaxa*. 2010;2612(1):22–40.
- Massa AM, Hozbor NM, Lasta C. Conditions of the coastal region of Bonaerense and Uruguay. Analysis of abundance and density estimates. *Inf Tec INIDEP N°54*. 2001;8.
- Massa AM, Lucifora LO, Hozbor NM. *Condrictios of the coastal region of Bonaerense and Uruguay*. In: Sánchez RP, Bezzi SI, editors. *The Argentine Sea and its fishing resources*. Sea fish of interest to fish. Biological characteristics and evaluation of the developmental stage. INIDEP; 2004. pp. 85–99.
- Gosztonyi AE, Kuba L. Presence of *Squalus mitsukurii* and aspects of its biology and nature of *Squalus acanthias* (Chondrichthyes, Squalidae) in Argentine waters during the months of February and June-July 1983. *Maritime Front*. 1998;17:49–60.
- Menni RC, Lucifora LO. *Chondrichthyans of Argentina and Uruguay: working list*. ProBiota, FCNyM, UNLP, Technical-Didactic Series; 2007:1–15.
- Meneses P, Paesh L. Field guide for the identification of cartilaginous fishes in the Río de la Plata and the ocean front. *Maritime Front*. 2003;19:145–194.
- Figueroa DE. Illustrated key to Agnatha and Cartilaginous fishes of Argentina and Uruguay. In: Wöhler OC, Cedrola P, Cousseau MB, editors. *Contributions on the biology, fishing and marketing of tiburones in Argentina*. Federal Fishing Council, Buenos Aires; 2011:25–74.
- Colonello JH, Cortes F, Belleggia M, et al. Diversity of sharks of the genus *Squalus* in the Argentine-Uruguayan Common Fishing Zone. *Frente Marit*. 2016;24:125–138.
- Andreoli G, Trucco MI. Evaluation of different DNA extraction protocols in spinal muscles in *Mustelus Schmitti*. *Inf Invest INIDEP N°7/2012*. 2012;8.
- Ward R, Zemlak T, Innes B, et al. DNA barcoding Australia's fish species. *Philos Trans R Soc Lond B Biol Sci*. 2005;360:1847–1857.
- Hall TA. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucl Acids Symp Ser*. 1999;41:95–98.
- Ratnasingham S, Hebert PD. BOLD: the barcode of life data system. *Mol Ecol Notes*. 2007;7:355–364.
- Tamura K, Stecher G, Peterson D, et al. MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol Biol Evol*. 2013;30(12):2725–2729.
- Librado P, Rozas J. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics*. 2009;25(11):1451–1452.
- Bandelt HJ, Forster P, Rohlf A. Median-joining networks for inferring intraspecific phylogenies. *Mol Biol Evol*. 1999;16(1):37–48.

24. Fluxus Technology. Network 10.1. Network© Copyright Fluxus Technology Ltd; 1999-2023.
25. Viana ST, Carvalho MR, Gomes UL. Taxonomy and morphology of species of the genus *Squalus* Linnaeus, 1758 from the Southwestern Atlantic Ocean (*Chondrichthyes: Squaliformes: Squalidae*). *Zootaxa*. 2016;4133(1):1–89.
26. Ariza AA, Adachi A, Roque P, et al. DNA barcoding and species delimitation for dogfish sharks belonging to the *Squalus* genus (*Squaliformes: squalidae*). *Diversity*. 2022;14(7):544.
27. Finucci B, Cheok J, Chiaramonte GE, et al. Spiny Dogfish (*Squalus acanthias*). The IUCN Red List of Threatened Species 2020: e.T91209505A124551959.
28. Cotton CF, Derrick D, Herman K, et al. Cuban Dogfish (*Squalus cubensis*). The IUCN Red List of Threatened Species 2020: e.T61416A3104105.
29. Cuevas JM, Michelson AM. Current state of knowledge on chondrichthyans in the Bahía San Blas multiple use natural reserve, province of Buenos Aires. 2023.