

Article

Integrating Egg Case Morphology and DNA Barcoding to Discriminate South American Catsharks, *Schroederichthys bivius* and *S. chilensis* (Carcharhiniformes: Atelomycteridae)

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Abstract

Catsharks are benthic elasmobranchs that share spatial niches with littoral and demersal bony fishes. The genus *Schroederichthys* includes five species, two of which, *S. chilensis* and *S. bivius*, occur in the waters of Chile. These species are morphologically similar and are often misidentified because of their overlapping external features and color patterns. To improve species discrimination, we analyzed the egg case morphology of both species based on 36 egg cases (12 *S. chilensis*, 24 *S. bivius*) collected from gravid females captured as bycatch in artisanal fisheries between Iquique and Puerto Montt (July–December 2021). Nine morphometric variables were measured and standardized using the total egg case length. Although the egg cases were similar in general appearance, multivariate analyses revealed significant interspecific differences, with egg case height and anterior border width emerging as the most diagnostic variables. Linear discriminant analysis achieved a 100% classification accuracy within this dataset. To confirm species identity, 24 tissue samples (12 per species) were sequenced for the mitochondrial cytochrome c oxidase subunit I (COI) gene. The haplotypes corresponded to previously published sequences from Chile (*S. chilensis*) and Argentina (*S. bivius*), with reciprocal monophyly and 100% bootstrap support. While COI barcoding provided robust confirmation, the core contribution of this study lies in the identification of species-specific egg case morphometrics. Together, these findings establish a dual-track toolkit, egg case morphology for primary discrimination and COI barcodes for confirmatory validation, that can be incorporated into bycatch monitoring and biodiversity assessments, supporting the conservation of poorly known catsharks in the Southeast Pacific.

Keywords: egg capsule; DNA barcoding; pintarroja; oviparous sharks



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1. Introduction

Colored catsharks (Carcharhiniformes: Atelomycteridae) are benthic elasmobranchs that share spatial niches with littoral and demersal bony fishes such as soles, hakes, and eels. However, unlike most bony fishes, catsharks possess life-history traits such as low fecundity, late sexual maturity, and relatively high longevity, which render them particularly vulnerable to overfishing [1]. These characteristics present significant conservation

challenges [2]. Over the past two decades, global assessments of shark conservation status have highlighted the susceptibility of small-bodied species to threats posed by incidental capture in fisheries [3], with some populations facing serious risks of depletion or local extinction [4].

Within Atelomycteridae, the subfamily Schroederichthyinae comprises six species, five of which are restricted to the Atlantic and Pacific coasts of Central and South America, and the recently described *Akheilos suwartanai* White, Fahmi, Weigmann, 2019, from Indonesia, which extends the subfamily beyond the Americas [5]. The genus *Schroederichthys* includes *S. chilensis* (Guichenot, 1848), distributed from southern Peru to central Chile; *S. biviuis* (Smith, 1838), occurring from southern Chile to northern Argentina; *S. maculatus* (Springer, 1966) from the western Atlantic; *S. saurisqualus* (Soto, 2001) from southern Brazil; and *S. tenuis* (Springer, 1966) from northern Brazil to Suriname [6–8]. In Chilean waters, *S. chilensis* and *S. biviuis* are thought to occur sympatrically over part of their latitudinal range, although they differ in depth preference: *S. chilensis* occupies subtidal rocky reefs between the Paracas Peninsula (14° S) and Chiloé Island (42° S), whereas *S. biviuis* occurs on the upper continental slope between Valdivia (39° S) and the Magallanes region (54° S), and potentially extends east into the southwestern Atlantic [9].

Although catsharks are relatively abundant along the Chilean coast [10,11], there is a lack of biological and taxonomic data on *S. chilensis*, and the available information on *S. biviuis* is geographically restricted, with studies focused primarily on Argentina [12,13]. Morphological differentiation between the two species is subtle and based on external features, such as body proportions, nasal flap shape, and dorsal coloration [14]. However, these characteristics are often obscured by phenotypic plasticity, ontogeny, and sexual dimorphism [13], making accurate identification challenging without comparative materials. Springer [14] noted in his global review of catsharks that “it has never been clearly and exhaustively established how to differentiate specimens of *S. chilensis* from *S. biviuis*,” a taxonomic uncertainty that persists to this day. Early anatomical studies also suggested intrageneric heterogeneity within the genus [6], a view reinforced by White et al. [5] and Soares and Mathubara [15], who argued that *S. biviuis* and *S. chilensis* may represent morphologically distinct lineages within the Schroederichthyinae.

Egg case morphology has long been recognized as a valuable character system in elasmobranch taxonomy. These structures frequently exhibit species-specific variations in size, shape, surface features, and pigmentation [16–19] and have been used in a wide range of studies, from reproductive ecology to phylogenetic inference [20–25]. Molecular tools, particularly mitochondrial DNA barcoding of the cytochrome c oxidase subunit I (COI) gene, have emerged as robust complementary methods for species identification [26–29].

For *Schroederichthys chilensis* and *S. biviuis*, whose overlapping external morphologies often hinder identification, COI barcoding may provide an independent line of evidence to support species delimitation. When integrated with egg case morphometrics, molecular data can significantly improve taxonomic resolution in ecological and fisheries contexts, particularly in cases where adult specimens are unavailable or damaged. Given the current lack of comparative information on egg case morphology and genetic variation in South American catsharks, this study aimed to evaluate the diagnostic value of egg case morphometrics and mitochondrial COI sequences in distinguishing *S. chilensis* from *S. biviuis*. This dual framework may provide a baseline for accurate species identification and offers practical tools for fisheries bycatch monitoring, biodiversity assessment, and conservation planning in the Southeast Pacific.

2. Materials and Methods

A total of 36 egg cases from *Schroederichthys chilensis* ($n = 12$) and *S. biviuis* ($n = 24$) were collected from the uteri of 18 individuals incidentally captured as bycatch in artisanal gillnet and longline fisheries along the Chilean coast. Between July and December 2021, specimens of *S. chilensis* were obtained from Iquique (20° S) and Valparaíso (33° S), and *S. biviuis* was obtained from Puerto Montt (41° S). For each egg case, the identity of the corresponding female was confirmed using the external diagnostic characters described by Springer [14] and Lamilla and Bustamante [30]. The uterus of origin (left or right) was recorded to test for potential intraspecific morphological differences and rule out any confounding effects on species discrimination. The egg cases were preserved in 70% ethanol for subsequent morphological analysis. In parallel, muscle tissue samples were collected from all gravid females and preserved in 90% ethanol for molecular analysis.

Egg case morphological terminology followed the established methodologies described in the literature [19,21,24]. Nine morphometric variables were recorded for each egg case (Figure 1), including total egg case length (L_{EC}), egg case height (H_{EC}), lateral keel length (LK), anterior border width (W_{AB}), anterior case width (W_{AC}), anterior waist width (W_{AW}), posterior base width (W_{PB}), posterior case width (W_{PC}), and posterior waist width (W_{PW}). Additional measurements of the anterior and posterior length of tendrils (L_{TE}) and the respiratory fissures (RF) were recorded but excluded from comparative analyses because tendrils are prone to natural breakage, which compromises measurement replicability, and the observation of respiratory fissures is highly subjective, limiting the consistency of this variable. All measurements were recorded to the nearest 0.01 mm using a digital caliper and expressed as proportions of L_{EC} to minimize size-related effects. Values are reported as mean $\pm$ standard deviation (s.d.). The spatial orientation of each egg (anterior and dorsal surfaces) was determined based on its position within the uterus, following the criteria described by Gomes and de Carvalho [31]. An independent two-sample t -test was conducted to evaluate potential differences in egg case length (L_{EC}) and posterior case width (W_{PC}) between the species (*S. chilensis* vs. *S. biviuis*). To compare the differences in L_{EC} and W_{PC} between the left and right uteri within individual females, a paired t -test was used following confirmation of normality and homogeneity of variances [32].

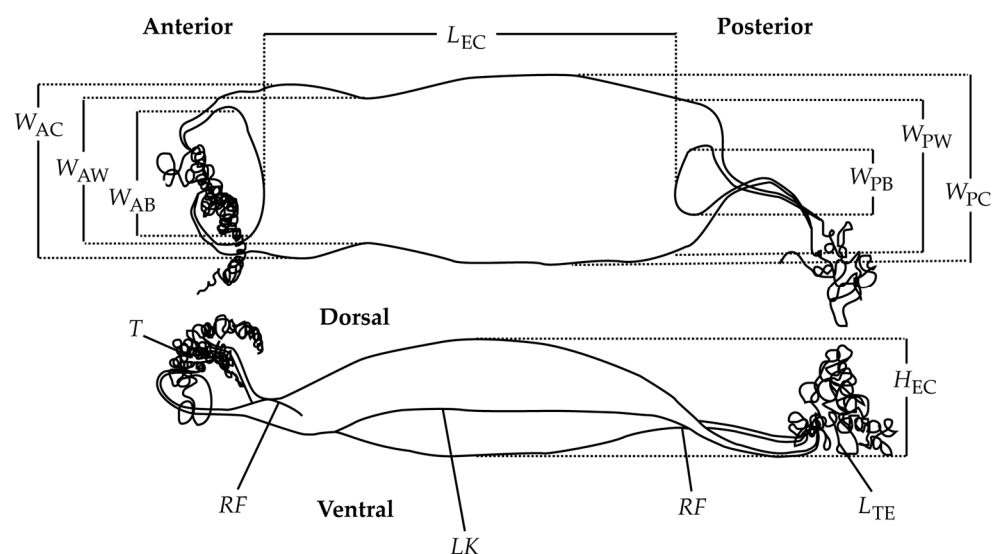


Figure 1. Schematic representation of a catshark-type egg case, showing measurements from the dorsal and lateral views. Abbreviations represent egg case length (L_{EC}), egg case height (H_{EC}), lateral keels (LK), anterior border width (W_{AB}), anterior case width (W_{AC}), anterior waist width (W_{AW}), posterior base width (W_{PB}), posterior case width (W_{PC}) and posterior waist width (W_{PW}), tendrils (T), length of tendrils (L_{TE}) and respiratory fissures (RF). Adapted from Bustamante et al. [24].

Morphometric measurements were used to characterize each egg case, with the identity of the egg-bearing female included as a grouping factor to account for potential non-independence among samples. Analyses were conducted in RStudio (version 2025.09.0) using the MorphoTools2 package (version 1.0.2.1) [33]. The normality of each variable was assessed using the Shapiro–Wilk test, and the homogeneity of variance was tested using Levene’s test. Variables were log-transformed where necessary to meet the assumptions of the parametric analysis. To identify and reduce redundancy among variables, Pearson’s correlation coefficients were calculated, and highly correlated pairs ($|r| \geq 0.95$) were excluded from analysis. The resulting dataset was used to construct a Euclidean distance matrix for hierarchical clustering and principal component analysis (PCA), allowing for a visual assessment of morphometric similarity among specimens. Linear discriminant analysis (LDA) was performed to assess the discriminatory power of the retained variables [34], generating discriminant functions that maximized the separation between the predefined groups. The assumption of homogeneity of the covariance matrices was evaluated using Box’s *M* test [33]. These analyses facilitated the identification of distinct morphotypes associated with species identity. To validate the classification performance, a cross-validation procedure was performed using the *k*-nearest neighbor (*k*-NN) method, which reassigns each specimen to its most likely group based on its morphometric profile and proximity to the group centroids.

Total genomic DNA was extracted from 24 individuals (12 *S. chilensis* and 12 *S. bivius*) using the standard phenol–chloroform protocol [35]. These samples corresponded directly to the gravid females from which egg cases were obtained, establishing a one-to-one correspondence between the morphological and molecular datasets. A fragment of the mitochondrial cytochrome c oxidase subunit I (COI) gene was amplified using the universal primers described by Ward et al. [36], as follows: PCR amplifications were carried out in 10 µL reactions containing 1 µL of genomic DNA (20–50 ng), 5.9 µL of Milli-Q H₂O, 1 µL of 10× buffer with MgCl₂ (15 mM), 1 µL of dNTPs (2 mM), 0.5 µL of each primer, and 0.1 µL of Taq DNA polymerase (5 U/µL). The thermal cycling profile consisted of an initial denaturation step at 95 °C for 2 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 54 °C for 30 s, and extension at 72 °C for 1 min, with a final extension step at 72 °C for 10 min. Amplified PCR products were purified using ExoSAP-IT (USB Products, Affymetrix, Inc., Santa Clara, CA, USA) by incubation at 37 °C for 45 min, followed by enzyme inactivation at 80 °C for 15 min. Sequencing was performed bidirectionally at the Austral-Omics Sequencing Facility (Valdivia, Chile). Sequence chromatograms were manually checked for base-calling errors, and variable sites were verified by comparing forward and reverse reads. Primer sequences were trimmed, and consensus sequences were assembled for each individual. Intraspecific genetic distances (*p*) were estimated using pairwise comparisons under the Kimura 2-parameter (K2P) model [37] in MEGA v7 [38]. The K2P distance matrix was also used to construct a neighbor-joining (NJ) tree in GENEIOUS [39], with node support assessed using 1000 bootstrap replicates. Novel haplotypes were deposited in the NCBI GenBank database under the accession numbers PX250298–PX250321. While GenBank remains a repository for homologous sequences of *Schroederichthys*, we acknowledge the risk of misidentified entries and therefore relied on previously validated haplotypes from Chile and Argentina to ensure confidence in species identity. The final alignments for COI included additional Scyliorhinidae species as outgroups to provide a phylogenetic framework for species discrimination (Table 1).

Table 1. List of colored catsharks (Atelomycteridae) and related species within the Carcharhiniiformes (including the outgroup) used in this study. The COI sequence accession numbers from the NCBI GenBank database are indicated. Novel sequences are indicated in bold.

Species	Locality	Accession Number
Carcharhiniiformes: Atelomycteridae		
<i>Atelomycterus baliensis</i>	Indonesia: Bali	EU398568, EU398569
<i>Atelomycterus erdmanni</i>	Indonesia	KP769787
<i>Atelomycterus fasciatus</i>	Australia: Western Australia	EU398570
<i>Atelomycterus marmoratus</i>	Philippines: Cebu	OQ386292, OQ386496
<i>Atelomycterus marnkalha</i>	Australia: Queensland	EU398574, EU398577
<i>Aulohalaelurus labiosus</i>	Australia: Western Australia	EU398581, HQ955988, JN312813
<i>Schroederichthys bivius</i>	Argentina	EU074581–EU074586
<i>Schroederichthys bivius</i>	Chile: Valdivia	PX250298–PX250303
<i>Schroederichthys bivius</i>	Chile: Puerto Montt	PX250304–PX250309
<i>Schroederichthys chilensis</i>	Chile: Coquimbo	MK982882, MK982902
<i>Schroederichthys chilensis</i>	Chile: Iquique	PX250310, PX250311
<i>Schroederichthys chilensis</i>	Chile: Valparaíso	PX250312–PX250315
<i>Schroederichthys chilensis</i>	Chile: San Antonio	PX250316–PX250321
Carcharhiniiformes: Scyliorhinidae		
<i>Cephaloscyllium laticeps</i>	Australia: Victoria	HQ956280
<i>Cephaloscyllium pictum</i>	Indonesia: Nusa Tenggara Barat	EU398676
<i>Cephaloscyllium silasi</i>	India	KF899711
<i>Cephaloscyllium variegatum</i>	Australia: Tasmania	HQ956282
<i>Poroderma africanum</i>	South Africa: Western Cape	OR138385, OR138381
<i>Poroderma pantherinum</i>	South Africa: Western Cape	OR138386, OR138391
<i>Scyliorhinus canicula</i>	USA: Washington	KJ709897, JN641232
<i>Scyliorhinus capensis</i>	South Africa: Western Cape	OR138392
<i>Scyliorhinus stellaris</i>	Malta	KJ709900
Carcharhiniiformes: Pentanchidae		
<i>Apristurus brunneus</i>	USA: Washington	JQ353982
<i>Apristurus melanoasper</i>	Canada: Newfoundland	MW339366
<i>Apristurus nasutus</i>	Chile: Coquimbo	KU737638
<i>Apristurus profundorum</i>	Canada: Newfoundland	MW339382
<i>Holohalaelurus punctatus</i>	South Africa	OR138367, OR138368
<i>Holohalaelurus regani</i>	South Africa: Western Cape	OR138372, OR138373
<i>Asymbolus analis</i>	Australia	HM902609
<i>Asymbolus parvus</i>	Australia: Western Australia	EU398564
<i>Asymbolus rubiginosus</i>	Australia	EU398567
Carcharhiniiformes: Carcharhinidae		
<i>Carcharhinus brachyurus</i> ¹	South Korea: Jeju island	MT995631

¹ Species included as an outgroup.

3. Results

3.1. Description of the Egg Cases

The egg cases of *Schroederichthys chilensis* (Figure 2a,b; Table 2) were relatively small, with a pronounced anterior waist and no horns or tendrils at the anterior end of the egg case. The surface was smooth on both the dorsal and ventral faces and was covered with a thin layer of fine filaments. The overall color was light brown. Near the anterior respiratory fissures, a bundle of fibrils originated that matched the thickness of the posterior horns. Posteriorly, the egg cases exhibited two well-developed horns, each giving rise to a long, robust tendril. These tendrils gradually tapered, reaching approximately one-third of their original diameter at the distal end of the tendril.

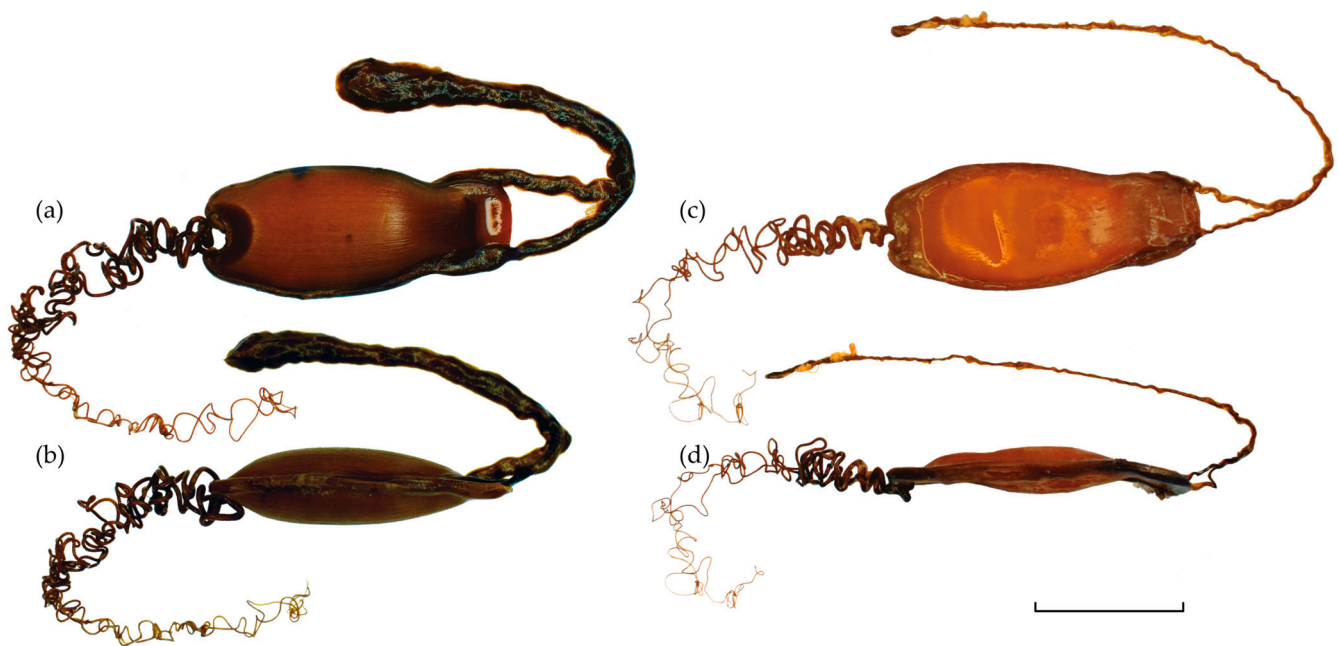


Figure 2. Egg cases of Chilean catsharks: (a,b) *Schroederichthys chilensis* and (c,d) *S. biviuis* in the dorsal (a,c) and (b,d) lateral views. The scale bar represents a length of 20 mm.

Table 2. Measurements of the egg cases of *Schroederichthys chilensis* and *S. biviuis* from Chilean waters. The mean values and standard deviations are expressed as percentages of L_{EC} . The abbreviations used for each measurement are presented in Figure 1.

Measurement	<i>Schroederichthys chilensis</i>		<i>Schroederichthys biviuis</i>	
	Mean (s.d.)	Range (mm)	Mean (s.d.)	Range (mm)
L_{EC}	52.3 (3.8)	45.2–57.1	52.8 (3.2)	45.0–58.6
H_{EC}	11.0 (1.8)	7.8–13.7	4.9 (1.6)	2.5–10.0
LK	3.3 (0.4)	2.8–4.0	2.0 (0.9)	0.1–3.7
W_{AB}	14.0 (0.4)	13.3–14.6	9.1 (1.3)	7.2–13.4
W_{AC}	17.4 (0.9)	15.7–18.5	13.0 (1.5)	10.2–16.6
W_{AW}	16.9 (1.1)	14.9–18.2	14.9 (1.1)	13.0–18.1
W_{PB}	5.8 (1.3)	4.1–7.7	6.7 (1.8)	3.2–9.2
W_{PC}	24.2 (1.7)	21.2–26.2	19.7 (2.3)	12.2–23.8
W_{PW}	18.7 (1.5)	16.1–20.3	13.7 (2.0)	10.7–18.1

The egg cases of *S. biviuis* (Figure 2c,d; Table 2) were similar in general shape and size to those of *S. chilensis* but had several distinguishing features. Like *S. chilensis*, the anterior end of *S. biviuis* egg cases lacked horns and tendrils; however, they displayed a characteristic double waist at the anterior margin. The color was light brown, and the surface texture was smooth. Fine filaments formed elongated thread-like cords near the anterior respiratory fissures. The posterior horns gave rise to tendrils that narrowed abruptly, tapering to approximately one-third of the egg case length before terminating in delicate filamentous tips.

3.2. Morphometric Analyses

All morphometric variables met the assumption of normality, as assessed using the Shapiro–Wilk test ($p > 0.05$). Within each species, no significant differences were detected in egg case length (L_{EC}) or posterior case width (W_{PC}) between the left and right uteri, indicating the absence of intra-individual asymmetry in these measurements. For *S. biviuis*, paired t -tests showed no significant differences in L_{EC} ($t = -0.0916$, $p = 0.8872$) or W_{PC} ($t = -1.0162$, $p = 0.3313$), and for *S. chilensis*, the results were similarly non-significant (L_{EC} :

$t = -0.6094$, $p = 0.8125$; W_{PC} : $t = 1.4363$, $p = 0.2104$). Interspecific comparisons revealed a significant difference in posterior case width, with *S. chilensis* exhibiting broader egg cases than *S. biviuis* ($t = 5.952$, $p < 0.001$). No significant difference was observed in the egg case length between species ($t = 0.5223$, $p = 0.604$). All morphometric variables met the assumptions of normality (Shapiro–Wilk test, $p > 0.05$) and homogeneity of variance (Levene’s test, $p > 0.05$). Following correlation filtering, eight variables were retained for multivariate analyses. Hierarchical clustering of Euclidean distance values revealed two non-overlapping groups corresponding to the two species (Figure 3). This strong species-level separation was consistent across individuals. The mean intraspecific dissimilarity was low (1.1–1.3 standardized Euclidean units), whereas interspecific dissimilarity was comparatively high (4.9 standardized Euclidean units), confirming a clear morphometric divergence between the two species.

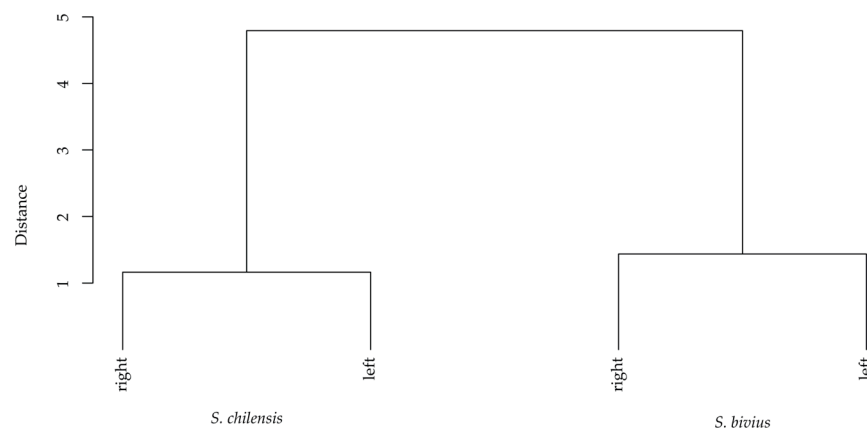


Figure 3. Comparative analysis of the left and right egg cases (associated with the uteri) of Chilean catsharks (*Schroederichthys chilensis* and *S. biviuis*) based on the Euclidean distance of eight morphometric variables standardized by the total egg case length.

The PCA ordination revealed a clear separation between *S. biviuis* and *S. chilensis* along the first two principal components, with PC1 and PC2 explaining 67.84% and 12.88% of the total variance, respectively (Figure 4a). The eigenvector biplot (Figure 4b) shows that the most influential variables along PC1 included anterior case width (W_{AC}), lateral keel length (LK), egg case height (H_{EC}), anterior border width (W_{AB}), and posterior case width (W_{PC}), all of which contributed to the species-level differentiation. Linear discriminant analysis (LDA) further supported species discrimination. Box’s M -test indicated homogeneity of covariance matrices ($\chi^2 = 2.72$, $df = 3$, $p = 0.437$). Two variables, W_{AB} and H_{EC} , significantly contributed to group separation. The classification model correctly assigned all egg cases to their respective species, achieving 100% classification accuracy within this dataset. All *S. biviuis* ($n = 24$) and *S. chilensis* ($n = 12$) egg cases were accurately classified based on the retained morphometric variables (Table 3). The k -nearest neighbor classification analysis showed that the highest number of correct classifications was at $k = 3$ and reflected the LDA results.

Table 3. Species classifications (‘as.’, e.g., as. *S. chilensis*) of Chilean catsharks (*Schroederichthys chilensis* and *S. biviuis*) by using a linear discriminant analysis (LDA) based on eight egg case morphometric measurements.

Taxon	<i>n</i>	as. <i>S. chilensis</i>	as. <i>S. biviuis</i>	<i>n</i> Correct	Percentage Correct
<i>S. chilensis</i>	12	12	0	12	100
<i>S. biviuis</i>	24	0	24	24	100
Total	36	12	24	36	100

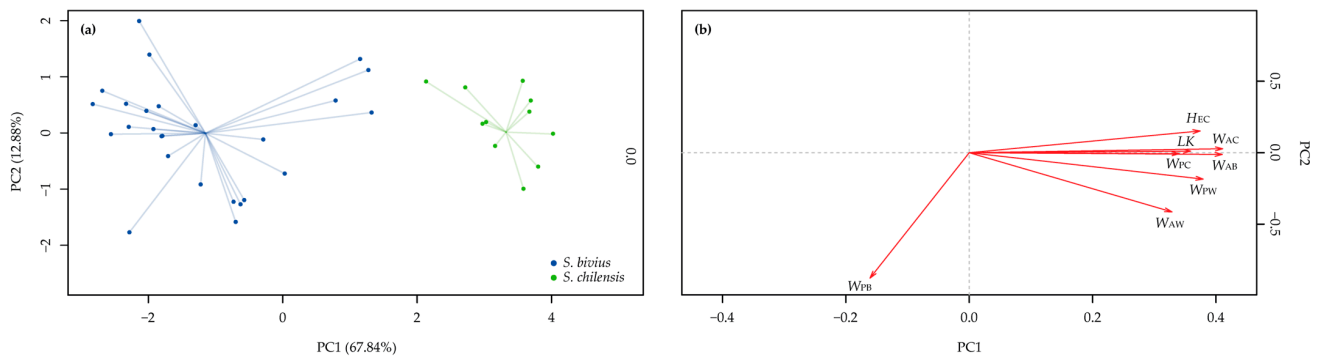


Figure 4. Principal component analysis (a) and eigenvector biplot (b) of the morphometric analysis of egg cases from Chilean catsharks (*Schroederichthys chilensis* and *S. biviuis*) based on the Euclidean distance of eight morphometric variables standardized by the total egg case length.

3.3. Molecular Analyses

A total of 24 high-quality COI sequences were obtained (12 *S. chilensis*, 12 *S. biviuis*), corresponding directly to the gravid females from which egg cases were analyzed. The sequence lengths ranged from 685 to 763 bp after trimming. No insertions, deletions, or stop codons were observed. Intraspecific K2P genetic distances were low (0.0021 for *S. chilensis*, 0.0018 for *S. biviuis*), whereas interspecific divergence was 0.053, consistent with species-level separation in elasmobranchs. Two haplotypes were detected in *S. biviuis* and one in *S. chilensis*, all of which matched previously published sequences from Argentina and Chile, respectively. Phylogenetic comparisons were performed using 67 sequences and 30 closely related species. The neighbor-joining tree based on K2P distances showed two well-supported clades corresponding to *S. chilensis* and *S. biviuis*, with 100% bootstrap support (Figure 5). No haplotypes were shared among the species. All sequences were grouped with reference sequences, confirming the correct species assignment and reflecting the current taxonomy of the Atelomycteridae family.

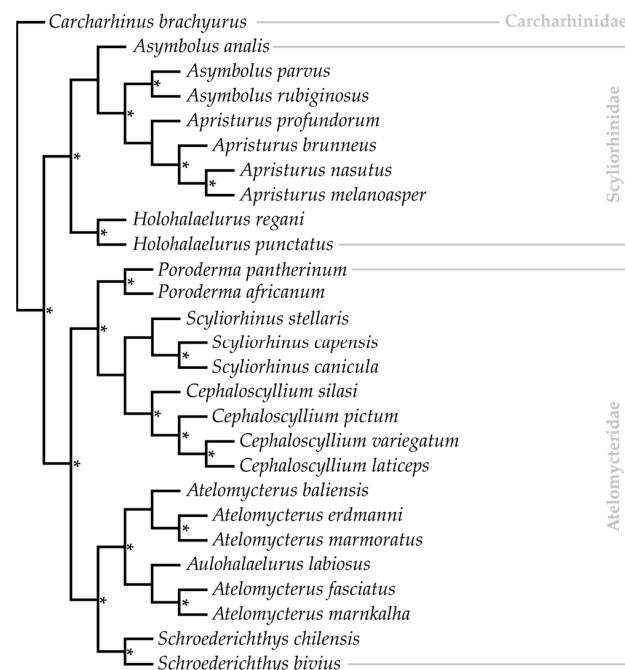


Figure 5. Phylogenetic tree of Chilean catsharks (Carcharhiniformes: Atelomycteridae) inferred from novel and reference COI sequences using a Neighbor-Joining (NJ) tree based on K2P parameter distances. The species *Carcharhinus brachyurus* was used as an outgroup representative to root the tree. Bootstrap support at branch nodes is shown (with a *) when > 80%. Families are represented outside the tip labels of the tree.

4. Discussion

This study provides an integrative approach to distinguish between two morphologically similar catshark species, *Schroederichthys bivi* and *S. chilensis*, using a combination of egg case morphometrics and mitochondrial DNA barcoding. Although both species produce externally similar egg cases, quantitative analyses revealed consistent species-specific differences, with egg case height and anterior border width emerging as robust diagnostic traits for species identification. The strong discriminatory signal was confirmed by linear discriminant analysis, which achieved 100% classification accuracy within the dataset. These findings validate the strong diagnostic power of reproductive characteristics and highlight the utility of egg case morphology as a practical tool for taxonomic identification in the absence of adults in the population. While this study did not explore environmental drivers, it aligns with previous reports [40], which suggested that the tendril structure of *Schroederichthys* egg cases enhances attachment to the structurally complex kelps, such as *Lessonia trabeculata*. This substrate specificity likely exerts selective pressure on stable morphological traits, such as posterior tendrils and consistent egg case shape, supporting their use as reliable taxonomic markers. The observed interspecific differences in egg case morphometrics appear to be robust across various environmental conditions, reinforcing the value of these characteristics for species-level identification.

The COI barcoding provided independent confirmation of species identity, recovering two reciprocally monophyletic clades with strong bootstrap support and no haplotype sharing. Intraspecific divergence was low, whereas interspecific divergence (5.3%) was well within the range typically observed among closely related elasmobranch taxa [26–29]. The neighbor-joining phylogeny showed reciprocal monophyly with 100% bootstrap support and no shared haplotypes, supporting previous studies that used COI as a robust marker for species identification in elasmobranchs [41,42]. These findings reinforce the value of DNA barcoding as a confirmatory tool that reinforces morphological findings, particularly in taxonomically challenging groups such as scyliorhinids [43–45]. In this study, COI barcoding was applied as a confirmatory marker because it remains the most widely applied and standardized barcode in vertebrate taxonomy. Although single-gene barcoding is limited in its phylogenetic resolution, it provides a robust baseline for species-level validation. Future studies should expand to multilocus or genomic approaches (e.g., NADH2, SNPs, RADseq) to test species boundaries across broader ranges and potential contact zones.

Egg case morphology has played an important role in elasmobranch taxonomy, and our findings add to the growing body of work that recognizes its value beyond reproductive biology. Previous studies have shown that the morphology of egg cases reflects both phylogenetic constraints and ecological adaptations [18,21]. Our comparative analysis confirmed the presence of stable, species-specific egg case features in South American catsharks and supports their continued use in systematic, evolutionary, and field-based identification studies of this group [43]. In practice, these morphometric characters may provide a rapid and non-lethal method for identifying species in fisheries bycatch, biodiversity surveys, and museum collections. When uncertainty persists, COI barcoding can be applied as a confirmatory layer, offering a straightforward workflow that integrates field identification and molecular verification. Such dual-track approaches can be readily adopted by fishery observer programs and national biodiversity monitoring initiatives, ensuring accurate species records even in the absence of adult specimens. Linking egg case occurrence with habitat information, such as the role of kelp forests as keystone habitats, further connects taxonomy to ecosystem-based management and conservation planning.

From a taxonomic perspective, our results are consistent with recent revisions of catshark systematics. Historically placed within Scyliorhinidae, recent systematic revi-

sions have recognized the family Atelomycteridae, with *Schroederichthyinae* as one of its subfamilies [5]. The description of *A. suwartanai* from Indonesia extends the distribution of this subfamily beyond South America, underscoring its previously underestimated diversity. Earlier anatomical studies also suggested that *S. bivius* and *S. chilensis* differ substantially from the “neotenic group” (*S. maculatus*, *S. saurissqualus*, and *S. tenuis*), raising the possibility that Chilean–Patagonian species represent a distinct lineage within the group [6,45]. Recently, Soares and Mathubara [15], using a robust phylogenetic framework based on 143 morphological traits and NADH2 gene sequences, confirmed the placement of *Schroederichthys* within Atelomycteridae and demonstrated that *S. bivius* and *S. chilensis* formed a well-supported monophyletic clade distinct from other scyliorhinids. Our results reinforce this updated classification, providing additional evidence from egg case morphology and mitochondrial COI data, highlighting the evolutionary distinctiveness of these species. The diagnostic egg case traits identified here are consistent with their separation from traditional Scyliorhinidae and support the monophyly of *Schroederichthys* within the Atelomycteridae.

Geographically, *S. bivius* and *S. chilensis* span broad latitudinal and bathymetric ranges along the southeastern Pacific, with a likely overlap between approximately 39°S (Valdivia) and 42°S (Chiloé Island). While *S. chilensis* is typically found in shallower subtidal zones, *S. bivius* occupies deeper shelves and upper continental slope habitats. However, field identification of either species remains difficult due to overlapping external features and high phenotypic plasticity in coloration and body proportions. Springer [14] originally distinguished *S. bivius* from *S. chilensis* by its pointed snout, narrower head, larger eyes, and longer nasal flaps. However, the diagnosis of these species remains incomplete and has not been comprehensively updated using modern morphometric or molecular tools.

Despite these advances, several limitations must be considered. Our sample sizes were modest, and the geographic coverage was restricted to Chilean waters. Future research should expand sampling across the full distributional range of both species, particularly in zones of potential sympatry. Additional studies should explore ontogenetic variation in egg-case morphology and incorporate environmental drivers (e.g., temperature and substrate) that may influence phenotypic traits. From a genetic perspective, multilocus or genome-wide approaches would strengthen species delimitation and test hypotheses, such as cryptic diversity or hybridization in contact zones.

Although *S. bivius* and *S. chilensis* are not currently the targets of commercial fisheries, they are often caught as bycatch in both artisanal and industrial fisheries along the Chilean coast [10]. However, no quantitative estimates of fishing mortality have been reported [11], leaving the impact on their populations unassessed. Given the low reproductive output and life-history traits of catsharks, such as late maturity, low fecundity, and long lifespan, unmonitored bycatch poses a clear conservation risk to these species [46,47]. These risks are exacerbated in coastal and demersal ecosystems, where habitat degradation compounds fishing mortality. Kelp forests, which dominate many subtidal zones along the Chilean coast, act as critical nursery and refuge habitats for diverse fish assemblages, enhancing the survival and recruitment of early life stages [48,49]. Therefore, the loss or fragmentation of these foundational habitats may indirectly affect the persistence of small benthic elasmobranchs, including *Schroederichthys* species, by altering prey availability and reducing shelter from predators [50]. In this context, the integrative framework developed here provides operational tools for monitoring and conservation efforts. Morphometric analysis of egg cases offers a rapid and non-invasive method for identifying species in the field, whereas COI barcoding provides a confirmatory layer when morphology alone is inconclusive. Together, these approaches form a dual-track identification workflow that can be directly incorporated into bycatch observer programs, biodiversity surveys and museum

collections. Linking species-specific egg case occurrence with habitat information, such as the role of kelp forests as nurseries, connects species-level taxonomy with ecosystem-based management. As human activities intensify pressures on coastal ecosystems, adopting such integrated identification tools will be essential to strengthen biodiversity assessments, mitigate bycatch impacts, and guide effective conservation of South American catsharks in the Southeast Pacific.

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