



Leucism in a commercially important monotypic marine teleost, the Cobia *Rachycentron canadum* (Linnaeus, 1766)

Leucismo en un teleósteo marino monotípico de importancia comercial, la cobia *Rachycentron canadum* (Linnaeus, 1766)

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Abstract

We report the first confirmed case of leucism in wild cobia (*Rachycentron canadum*), a widely distributed and economically significant marine teleost, from the southwest coast of India. The specimen was captured near Kollam, Kerala, and exhibited extensive hypopigmentation across the body, with only faint dorsal markings and normally pigmented eyes, distinguishing it from albinism. Morphological identification was corroborated using standard taxonomic keys, and genetic analysis of the mitochondrial cytochrome c oxidase subunit I (COI) gene confirmed the specimen's identity with 100% sequence match to *R. canadum* reference sequences in GenBank and BOLD. Comparison with a normally pigmented individual highlighted the rarity and visual distinctiveness of this coloration anomaly. Leucism in marine fishes is uncommon and may arise due to genetic, environmental, or epigenetic factors, though the

► Ref. bibliográfica: John, S. P.; Morris, S.; Morris, S.; Joseph, P.; Gopi, L. 2026. "Leucism in a commercially important monotypic marine teleost, the Cobia *Rachycentron canadum* (Linnaeus, 1766)". *Acta Zoológica Lilloana* 70 (1): 245-264. DOI: <https://doi.org/10.30550/j.azl/2351>

► Recibido: 16 de enero 2026 – Aceptado: 17 de marzo 2026.

► URL de la revista: <http://actazoolologica.lillo.org.ar>

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exact cause in this case remains undetermined. This finding contributes to the growing documentation of pigmentary abnormalities in wild marine fishes and raises questions about the underlying mechanisms and potential ecological consequences. Future studies integrating broader genomic and ecological data are needed to elucidate the drivers of such rare phenotypes.

Keywords: Leucism; Cobia; *Rachycentron canadum*; Color anomaly; Hypomelanosis; Pigment deficiency; Morphological variation.

Resumen

Informamos del primer caso confirmado de leucismo en la cobia salvaje (*Rachycentron canadum*), un teleosteo marino de amplia distribución y de gran importancia económica, procedente de la costa suroeste de la India. El ejemplar fue capturado cerca de Kollam, Kerala, y presentaba una extensa hipopigmentación en todo el cuerpo, con solo leves marcas dorsales y ojos con pigmentación normal, lo que lo distingue del albinismo. La identificación morfológica se corroboró utilizando claves taxonómicas estándar, y el análisis genético del gen de la subunidad I de la citocromo c oxidasa mitocondrial (COI) confirmó la identidad del espécimen con una coincidencia de secuencia del 100 % con las secuencias de referencia de *R. canadum* en GenBank y BOLD. La comparación con un individuo de pigmentación normal puso de manifiesto la rareza y la singularidad visual de esta anomalía de coloración. El leucismo en peces marinos es poco común y puede deberse a factores genéticos, ambientales o epigenéticos, aunque la causa exacta en este caso aún no se ha determinado. Este hallazgo contribuye a la creciente documentación de anomalías pigmentarias en peces marinos silvestres y plantea preguntas sobre los mecanismos subyacentes y las posibles consecuencias ecológicas. Se necesitan estudios futuros que integren datos genómicos y ecológicos más amplios para dilucidar los factores que originan estos fenotipos poco frecuentes.

Palabras clave: Leucismo; Cobia; *Rachycentron canadum*; Anomalía de color; Hipomelanosis; Deficiencia de pigmentación; Variación morfológica.

INTRODUCTION

Coloration in fishes is highly diverse, varying across species, populations, and individuals, and plays vital roles in communication, camouflage, reproduction, social signaling, and predator avoidance (Liu et al., 2024; Üstündağ et al., 2019; Cal et al., 2018; Hubbard et al., 2010; Rodgers et al., 2010). Additionally, pigmentation contributes to protection from ultraviolet (UV) radiation, aids in sexual dimorphism, and is crucial for development and survival (Alves and Agusti, 2020; Icoğlu Aksakal and Ciltas, 2018; Vásquez et al., 2015; Mueller and Neuhauss, 2014).

Colour abnormalities are known to occur in all vertebrate classes: mammals (Martin et al., 2025; Hofmeester et al., 2021), amphibians (Almeida-Reinoso et al., 2023; Tavares-Pinheiro et al., 2020), reptiles (Park et al., 2024; Deshmukh et al., 2020), birds (Sarlin et al., 2025; Warner et al., 2024; van Grouw, 2021), fish (Campos-León et al., 2025; Ray et al., 2024; Fontes et al., 2023; González-Ortegón et al., 2020), but it is rare in natural populations (Camacho et al., 2022; Arronte et al., 2022). Albinism and leucism are truly rare in nature among chondrichthyan species and more frequent among teleosts (Arronte et al., 2022; Bigman et al., 2016; Diatta et al., 2013).

Thus, due to their rarity, it is important to document both wild and captive observations of skin aberrations to help understand color patterns and their biological significance across time, space, and taxa, and to help inform future observations (Skelton et al., 2024).

Fish pigmentation is controlled by various chromatophores: melanophores (black/brown), xanthophores (yellow), erythrophores (red), iridophores (reflective), cyanophores (blue), and leucophores (white) (Bagnara & Matsumoto, 2006; Kelsh, 2004; Bechtel, 1995). Disorders in these pigment cells may arise due to genetic mutations (Sandoval-Castillo et al., 2006; Clark, 2002), nutritional deficiencies (Peles et al., 1995), environmental stressors, or exposure to pollutants (Møller and Mousseau, 2001). Hypopigmented individuals often experience reduced fitness, increased predation risk, and impaired social signalling (Di Marzio and Rozentals, 2021; Bruni, 2017).

Cobia (*Rachycentron canadum*) (Linnaeus, 1766), the sole representative of Rachycentridae, is a large pelagic teleost distributed in tropical and subtropical regions worldwide, with high aquaculture and commercial value (dos Santos Júnior and de Nóbrega, 2024; Ganga et al., 2024). Although the names *Rachycentron blochii* and *Rachycentron makranesis* were recently proposed (Panhwar and Kashani, 2024), they are currently considered unavailable due to the absence of type designations (Froese and Pauly, 2026), and *R. canadum* remains the valid species. It is widely farmed in India due to its rapid growth and adaptability to cage culture. Genetic studies have identified three major populations along the Indian coast (Divya et al., 2017).

Cobia typically exhibits a dark dorsum with two darker horizontal bands and a white ventrum (Froese and Pauly, 2025; Shaffer and Nakamura, 1989). However, coloration can change based on mood or developmental stage (Su et al., 2000). Although morphometric analyses have revealed early sex shape dimorphism and sexually dimorphic growth in *cobia* (Molina et al., 2018; Dutney et al., 2017), sexual dimorphism is not strongly expressed externally (Benetti et al., 2021). Consequently, accurate sex determination at the individual level, particularly in wild specimens, remains challenging without dissection or genetic analysis.

As leucism may arise due to inbreeding, genetic mutations, or environmental stressors -including pollution and overfishing - its documentation in wild marine species is essential (González-Ortegón et al., 2020; Bruckner and Coward, 2018). Citizen science and observations by recreational fishers and divers can significantly contribute to monitoring these rare events (Schuhbauer et al., 2025). We document the first observation of a leucistic *R. canadum* specimen from Kerala, southwest India.

MATERIALS AND METHODS

Specimen Collection and Morphological Identification

A leucistic specimen of wild cobia was captured on 12 April 2024 from an offshore location (9.119370217547148, 75.1213019233708) along the southwest coast of India. The fish was landed dead at Vaddy Fishing Harbour, Kollam, Kerala (Figure 1), by a single-day longline fishing vessel. Morphological identification was conducted following established taxonomic keys (FAO, 2009; Fischer & Bianchi, 1984). Comparative morphological evaluation was carried out by referencing a size-matched normally pigmented conspecific specimen landed at the same site.

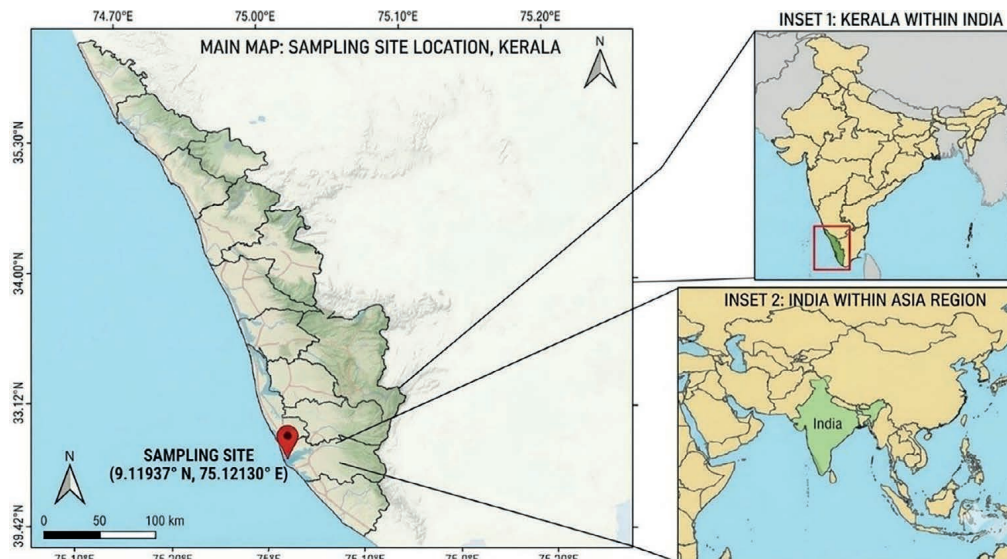


Figure 1. Map of study area.

Figura 1. Mapa del área de estudio.

DNA isolation, PCR amplification, and sequencing

Genomic DNA was isolated from muscle tissues of the leucistic specimen using the NucleoSpin® Tissue Kit (Macherey–Nagel), following the manufacturer's instructions. A fragment of the mitochondrial cytochrome c oxidase subunit I (COI) gene was amplified using the primer pair LCO (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO (5'-TAAACTTCAGGGTGACCAAAAAATCA-3'), as described by Ward et al., 2005.

Polymerase Chain Reaction (PCR) amplification was carried out on a Mastercycler PCR System (Eppendorf). The thermal cycling conditions consisted of an initial denaturation at 98 °C for 30 seconds, followed by 10 cycles of denaturation at 98 °C for 5 seconds, annealing at 50–72 °C for 15 seconds, and extension at 72 °C for 15 seconds, concluding with a final extension at 72 °C for 60 seconds (Hall et al., 1994).

PCR products were purified using ExoSAP-IT (GE Healthcare) to remove unincorporated primers and nucleotides. Sequencing was performed using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA) on a GeneAmp PCR System 9700 (Applied Biosystems), in accordance with the manufacturer's protocol.

The quality of the resulting sequences was evaluated using Sequence Scanner Software v1 (Applied Biosystems), and sequence editing and alignment were conducted with BioEdit version 7.0.5.2 (Hall, 1994). The edited DNA sequence (PV636261 - 586bp) was successfully submitted to the NCBI GenBank database.

Molecular phylogenetic analysis

To investigate the evolutionary relationships of *R. canadum*, one original COI (cytochrome c oxidase subunit I) gene sequences were generated and supplemented with 32 additional sequences retrieved from GenBank. This yielded a total of 33 sequences representing a diverse array of teleost taxa. Multiple sequence alignment was performed using the ClustalW algorithm implemented in MEGA12 software. Phylogenetic trees were constructed using the Maximum Likelihood (ML) method based on the Tamura-Nei substitution model, which was identified as the best-fit model for the dataset.

The phylogeny was inferred using the ML method and Tamura-Nei (1993) model of nucleotide substitutions and the tree with the highest log likelihood (-6,721.13) is shown. The percentage of replicate trees in which the associated taxa clustered together (1,000 replicates) is shown next to the branches (Felsenstein, 1985). The initial tree for the heuristic search was selected by choosing the tree with the superior log-likelihood between a Neighbor-Joining (NJ) tree (Saitou and Nei 1987) and a Maximum Parsimony (MP) tree.

The NJ tree was generated using a matrix of pairwise distances computed using the Tamura-Nei (1993) model. The MP tree had the shortest length among 10 MP tree searches; each performed with a randomly generated starting tree. The analytical procedure encompassed 33 coding nucleotide sequences using 1st, 2nd, 3rd, and non-coding positions with 586 positions in the final dataset. Evolutionary analyses were conducted in MEGA12 (Kumar et al., 2024) utilizing up to 4 parallel computing threads.

The best fit nucleotide substitution model for the aligned sequences of *R. canadum* was determined using the model selection function implemented in MEGA 12, which evaluates multiple candidate models based on likelihood scores and information criteria. Among the tested models, the Tamura-Nei model showed the lowest Bayesian Information Criterion (BIC) value and was therefore selected as the best-fit model for ML phylogenetic reconstruction.

The ML tree inference employed the Nearest-Neighbor Interchange (NNI) heuristic search method. Bootstrap analysis with 1000 replicates was performed to assess the reliability and statistical support of the tree nodes. To root the phylogenetic tree, sequences from *Ambloplites ariommus* (JN024727) and *Ambloplites rupestris* (MT150955) were used as outgroup taxa, representing a basal lineage relative to the ingroup.

A total of **33 COI gene sequences** were analyzed, comprising the **original** *R. canadum* sequences generated for this study and **32 additional sequences** representing a broad range of teleost fish taxa obtained from the NCBI GenBank database. The dataset included members from multiple families, such as Carangidae, Coryphaenidae, Scombridae, and Echeneidae, to provide a comprehensive phylogenetic context. Six species *Pseudorhombus javanicus* JN313338, *Pseudorhombus jenynsii* OL307687, *Seriola dumerili* MZ436143, *Seriola lalandi* LC178081, *Seriola lalandi* JQ738432 were selected as **outgroup taxa** to root the tree appropriately.

RESULTS

The leucistic specimen of *R. canadum* measured 67 cm in total length and weighed 5.16 kg. Morphologically, it exhibited pronounced hypopigmentation across the body, consistent with classical leucism. The head and dorsum were yellowish-white, while the ventral surface appeared uniformly pale or cream-colored. The dorsal surface was uniformly yellowish, while the lateral and ventral surfaces were predominantly white or pink-colored. Scattered taupe-colored spots were present dorsally, and darker pigment was retained along the fin margins, particularly the second dorsal and caudal fins. The pectoral and pelvic fins displayed faint pinkish hues. Crucially, the eyes retained normal melanin pigmentation, distinguishing the condition from albinism. The fish showed no deformities, external injuries, or signs of impaired health or development Figure (2 a-d).

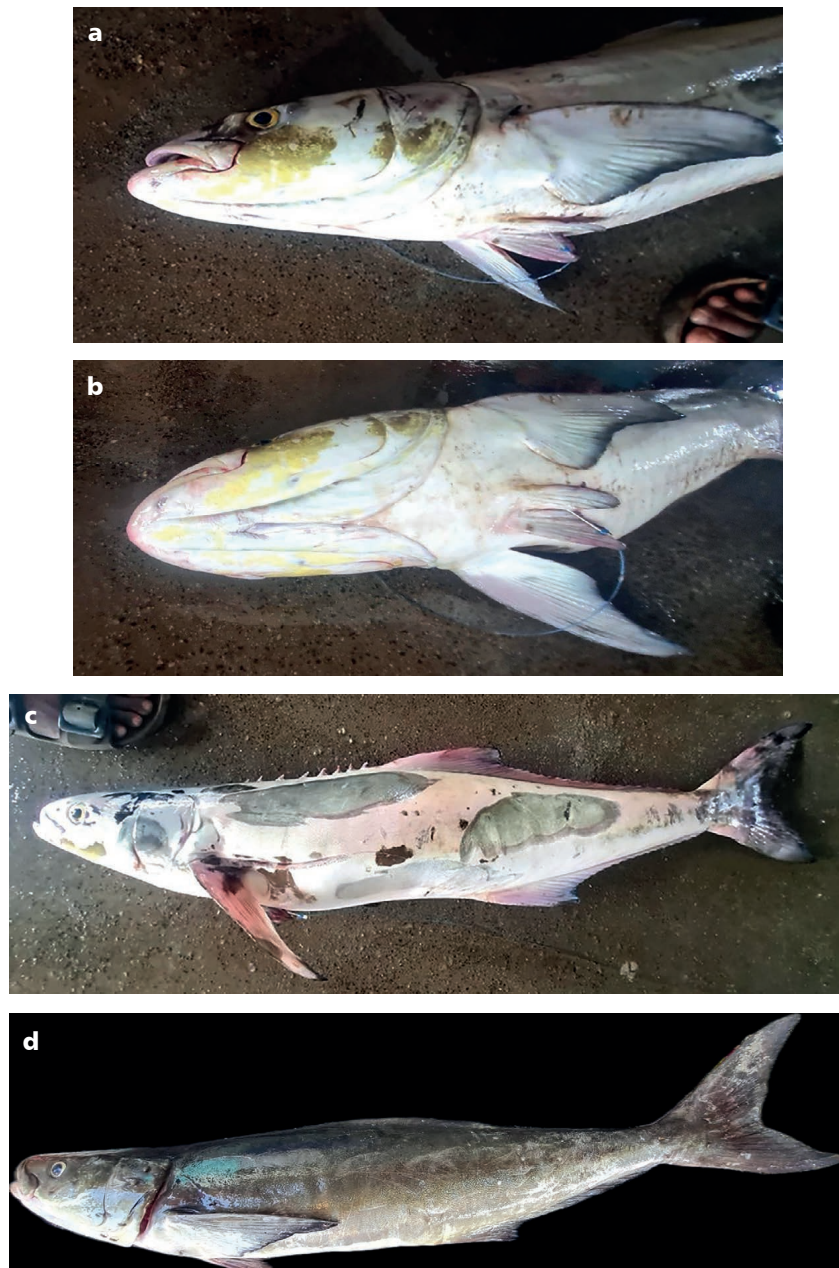


Figure 2. **a)** Close-up of the head region of the leucistic cobia, showing abnormal yellow pigmentation patterns and pinkish fin bases. **b)** Anterolateral view of the leucistic cobia's head, highlighting the pale skin, prominent eye, and distinct loss of melanin in the opercular and maxillary regions. **c)** Lateral view of the leucistic *R. canadum* specimen showing the pale coloration pattern and irregular dark blotches. The typical countershading seen in wild-type cobia is absent. **d)** A wild-type *R. canadum* specimen from the same landing site, showing normal dark coloration and countershading for comparison.

Figura 2. **a)** Primer plano de la región cefálica de la cobia leucística, que muestra patrones de pigmentación amarilla anormales y bases de las aletas rosadas. **b)** Vista anterolateral de la cabeza de la cobia leucística, donde se aprecian la piel pálida, el ojo prominente y la marcada pérdida de melanina en las regiones opercular y maxilar. **c)** Vista lateral del ejemplar leucístico de *R. canadum*, que muestra el patrón de coloración pálida y manchas oscuras irregulares. El sombreado típico observado en la cobia silvestre está ausente. **d)** Un ejemplar silvestre de *R. canadum* del mismo lugar de desembarque, que muestra la coloración oscura normal y el sombreado para comparación.

The resulting phylogenetic tree revealed several well-supported clades and clearly delineated relationships among the analyzed taxa: The two *R. canadum* sequences formed a monophyletic clade with 100% bootstrap support, confirming their close genetic relatedness and consistent identity as the same species. The sequence obtained by our study (GenBank accession number PV636261) was closely related to sequence MT076813 of the same species, further affirming the phylogenetic coherence of the genus. The *Rachycentron* clade clustered within a larger grouping that included *Coryphaena equiselis*, indicating a moderately close evolutionary relationship. Other taxa such as *Seriola lalandi*, *Seriola dumerili*, and *Scomberoides lysan* also formed strongly supported clades with bootstrap values of 100%. Outgroup taxa (*Ambloplites ariommus* and *Ambloplites rupestris*) appropriately rooted the tree and were clearly distinct from all ingroup members, validating the tree structure (Figure 3).

The apparent closer association of *Rachycentron canadum* MT076813 with *Trachinotus blochii* PQ380990 is best interpreted as sequence level variation rather than a true biological relationship. Because both genera fall within the order Carangiformes, some degree of clustering is expected when phylogenetic resolution is limited. In contrast, our sequence (PV636261) consistently groups in the expected position for *R. canadum*, supporting its correct taxonomic placement.

The high bootstrap support values across many nodes suggest a robust phylogenetic signal in the COI gene region. The placement of *R. canadum* as a distinct and well-supported lineage supports its current taxonomic status within the order Carangiformes and highlights the utility of COI barcoding in species-level identification and taxonomic resolution (Figure 4).

In contrast, the control specimen, a normally pigmented conspecific, exhibited the typical coloration of *R. canadum*: a dark brown to black dorsal surface, a white ventral surface, and a dense distribution of small dark spots, particularly near the posterior pectoral region, consistent with previously published descriptions (Nazar and Jayakumar, 2017).

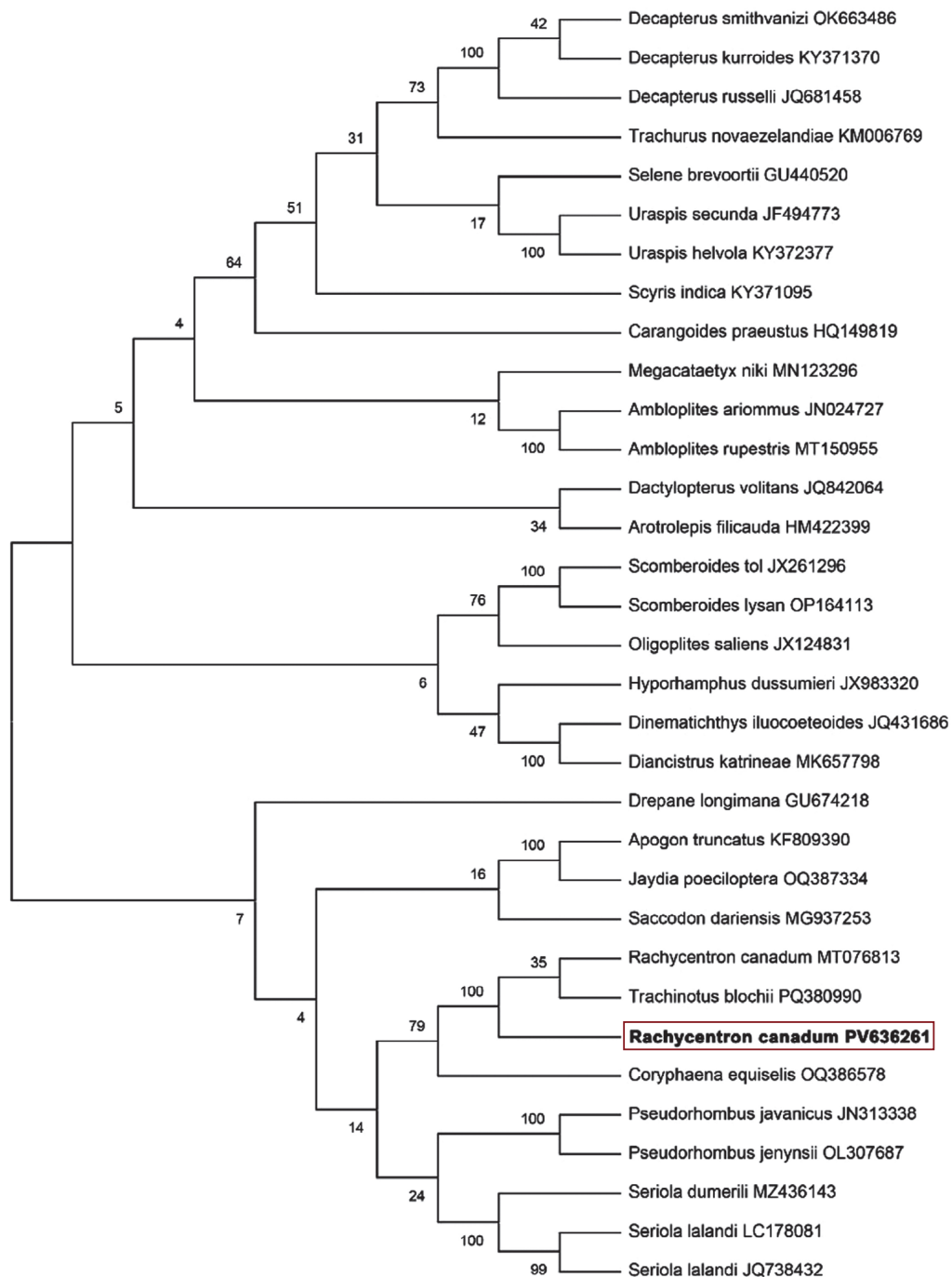


Figure 3. Phylogenetic tree showing the evolutionary relationships among *R. canadum* and related teleost species based on COI gene sequences. *R. canadum*, the species of interest in this study, is highlighted. The phylogenetic tree was inferred using the ML method based on the Tamura-Nei model. The values near the nodes represent bootstrap support percentages based on 1000 replicates, indicating the confidence level of each clade.

Figura 3. Árbol filogenético que muestra las relaciones evolutivas entre *R. canadum* y especies de teleosteos relacionadas, basado en secuencias del gen COI. *R. canadum*, la especie de interés en este estudio, se encuentra destacada. El árbol filogenético se infirió mediante el método de máxima verosimilitud (ML) basado en el modelo de Tamura-Nei. Los valores junto a los nodos representan los porcentajes de soporte bootstrap basados en 1000 réplicas, lo que indica el nivel de confianza de cada clado.

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PV636261   TCGGTGTCTTAGCCGGAATAACAGGAACAGGCCTAAGTCTCCTCATTGAGCAGAATTAA
MT076813   TCGGTGTCTTAGCCGGAATAACAGGAACAGGCCTAAGTCTCCTCATTGAGCAGAATTAA
*****
PV636261   GCCAACCTGGCTCCCTACTGGGAGACGACCAAACCTACAACGTAATCGTAACAGCCCACG
MT076813   GCCAACCTGGCTCCCTACTGGGAGACGACCAAACCTACAACGTAATCGTAACAGCCCACG
*****
PV636261   CCTTCGTAATAATCTTCTTTATAGTAATACCAATTATGATTGGAGGCTTTGGGAACTGAC
MT076813   CCTTCGTAATAATCTTCTTTATAGTAATACCAATTATGATTGGAGGCTTTGGGAACTGAC
*****
PV636261   TTATTCCTTAATGCTAGGCGCCCGATATGGCTTTTCCCGTATAAATAATATAAGTT
MT076813   TTATTCCTTAATGCTAGGCGCCCGATATGGCTTTTCCCGTATAAATAATATAAGTT
*****
PV636261   TCTGACTACTCCCCATCATTCTCTGCTGCTAGCCTTTTCAGGTGTTGAAGCTGGAG
MT076813   TCTGACTACTCCCCATCATTCTCTGCTGCTAGCCTTTTCAGGTGTTGAAGCTGGAG
*****
PV636261   CAGGGACTGGTTGGACAGTTTACCCACCTCTGGCGGGCAACCTAGCACATGCAGGAGCCT
MT076813   CAGGGACTGGTTGGACAGTTTACCCACCTCTGGCGGGCAACCTAGCACATGCAGGAGCCT
*****
PV636261   CTGTTGACTTAACTATTTTCTCCCTTCATCTTGCAGGGGTGTCTTCAATTCTCGGGGCTA
MT076813   CTGTTGACTTAACTATTTTCTCCCTTCATCTTGCAGGGGTGTCTTCAATTCTCGGGGCTA
*****
PV636261   TTAATTTTATTACAACAATTATTAACATAAAACCACCAACCGTGACTATGTACCAAATTC
MT076813   TTAATTTTATTACAACAATTATTAACATAAAACCACCAACCGTGACTATGTACCAAATTC
*****
PV636261   CCCTCTCGTATGGGCTGTCTAATCACTGCCGTCTTCTCCTCTCACTCCCAGTCC
MT076813   CCCTCTCGTATGGGCTGTCTAATCACTGCCGTCTTCTCCTCTCACTCCCAGTCC
*****
PV636261   TGGCTGCTGGCATTACTATACTGCTTACAGACCGAAATTTAAATAC
MT076813   TGGCTGCTGGCATTACTATACTGCTTACAGACCGAAATTTAAATAC
*****

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Figure 4. CLUSTAL 2.1. Multiple sequence alignment for specific SNP differences.

Figura 4. Alineamiento de secuencias múltiples para diferencias específicas de SNP.

DISCUSSION

Leucism, a pigmentation disorder characterized by reduced or absent melanin in the skin while retaining normal eye pigmentation, is rarely documented in marine teleosts and elasmobranchs (Campos-León et al., 2025; Arronte et al., 2022). The current record represents the first known observation of leucism in *R. canadum*.

Previous studies have reported leucism in a range of fish species, though such cases remain infrequent (Fontes et al., 2023; Bigman et al., 2016; Diatta et al., 2013). Contributing factors may include both genetic mutations and environmental triggers. Leucism has been linked to inheritable genetic anomalies, either homozygous or heterozygous mutations as well as to epigenetic responses to environmental variables such as diet, disease, injury, inbreeding, and temperature fluctuations (Espinal et al., 2016; Gervais et al., 2016; Sandoval-Castillo et al., 2006).

The coloration observed in this specimen - extensive hypopigmentation with residual pigment patches and normal eye color is consistent with classical leucism, as described by Clark (2002). The absence of albinism-related impairments such as visual deficits, poor body condition, or feeding anomalies further supports this classification. Similar pigment disorders have also been attributed to anthropogenic factors such as pollution, overfishing, and habitat degradation (Fontes et al., 2023; González-Ortegón et al., 2020; Bruckner and Coward, 2018).

In general, hypopigmented fishes are considered ecologically disadvantaged due to their increased visibility to predators, compromised camouflage, and susceptibility to ultraviolet radiation (Acevedo et al., 2009; Sandoval-Castillo et al., 2006; Uieda, 2000). These selective pressures likely contribute to the low frequency of leucistic individuals in natural populations. However, the presence of sexually mature leucistic fish in various taxa (Arronte et al., 2022; Becerril-García et al., 2017; Bottaro et al., 2008) indicates that this phenotype does not always inhibit survival or reproduction.

The specimen analyzed here showed no signs of compromised physiological function or competitive exclusion, aligning with prior reports that pigmentation anomalies do not necessarily impair ecological performance. Nevertheless, due to the potential costs associated with conspicuous phenotypes in visually structured marine environments, leucism is likely selected against in most wild populations (Sazima and Di Bernardo, 1991; Talent, 1973).

Pigmentation in fishes is regulated by a combination of hormonal, genetic, and environmental factors, and may change over the lifespan of an individual. Such changes may occur during life history transitions, reproductive cycles, or in response to biotic interactions and abiotic stressors such as ultraviolet radiation, ambient light levels, and temperature (Parichy and Spiewak, 2015; Darias et al., 2013a, b; Pittman et al., 2013; Leclercq et al., 2010; Price et al., 2008).

Although the precise cause of this leucism remains undetermined, it may result from multifactorial influences as suggested in earlier studies (Colmenero et al., 2015; Ebert, 1985; Herald, 1953). Small population sizes and genetic bottlenecks can increase the likelihood of recessive pigment mutations under anthropogenic pressures. While such cases have not been documented in cobia, similar patterns occur in other teleosts; for example, albinism in cave populations of *Astyanax mexicanus* is linked to mutations in pigmentation genes (e.g., *oca2*, *mc1r*) that became prevalent through genetic drift in isolated populations (Zhang et al., 2025). In cobia, anthropogenic influences such as mariculture escapes (Castellanos-Galindo et al., 2016) and sea-ranching initiatives in Kerala (Anilkumar, 2011) may influence population genetic structure, and hatchery-reared individuals released into the wild can be distinguishable from native fish (dos Santos et al., 2025).

However, although cobia populations off Kerala are genetically distinct from other Indian populations, no recent demographic bottlenecks have been detected (Divya et al., 2017), and thus any direct link between this genetic structure and leucism remains uncertain.

This study demonstrates that even rare pigment anomalies like leucism can offer valuable insights into fish biology, genetics, and environmental responses. Given the inherent difficulty in detecting such traits in wild marine fishes, incidental records from fisheries landings provide essential contributions to biodiversity documentation and phenotypic variability in marine species.

CONCLUSION

This study documents the first confirmed case of leucism in *R.canadum* from Indian waters, verified through both morphological assessment and COI gene sequencing. Despite the pronounced hypopigmentation, the specimen displayed no morphological or physiological abnormalities, indicating that leucism may not always compromise survival in marine teleosts. Given the rarity of such pigmentation anomalies, continued documentation and genetic validation of aberrant phenotypes are essential for advancing our understanding of pigmentation biology, population genetics, and potential environmental influences affecting wild fish populations.

ACKNOWLEDGEMENT

We sincerely thank Steve Lockett of Mahseer Trust for translating the abstract into Spanish.

DECLARATION OF GENERATIVE AI IN SCIENTIFIC WRITING

Generative AI tools were used only for language editing and improving clarity. All scientific content, analysis, and conclusions are the responsibility of the authors.

CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

Sandie Morris: Writing – review & editing, photography, measurements., Pathissery John Sarlin: Writing – review & editing, Visualization, Validation, Methodology, Investigation, Sancia Morris: Validation, Methodology, Investigation, writing, review & editing. Savio Morris: Writing – review & editing, photography, Methodology. Polycarp Joseph: Writing – original draft, Methodology, Investigation. Lijin Gopi: Data curation and Formal Analysis.

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.

FUNDING DECLARATION

No specific funding or financial support was received for this study. The authors acknowledge the infrastructural and research facilities provided to the Department of Zoology, Fatima Mata National College, Kollam, under the Department of Science and Technology–Fund for Improvement of S&T Infrastructure in Universities and Higher Educational Institutions (SR/FST/COLLEGE-327/2018), and thank the college management for their continued support.

ETHICAL APPROVAL

This study did not involve any experiments on live animals. The fish specimens were obtained from routine commercial fishing activities, and no animals were sacrificed specifically for the purposes of this research. Therefore, formal ethical approval was not required.

CONSENT TO PARTICIPATE

Not applicable, as the study did not involve human participants.

CONSENT TO PUBLISH

All authors have read and approved the final version of the manuscript and consent to its publication.

DATA AVAILABILITY

All data are contained within the manuscript.

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