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SHORT COMMUNICATION

Genetic and taxonomic data corroborate the existence of lemon shark, *Negaprion brevirostris* (Carchariniformes: Carcharhinidae) (Poey, 1868) in Coiba National Park, Panama

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Abstract

For almost 2 years community structure, characterization and identification of the main fish species from the largest estuaries of Coiba National Park have been the focus of our research interest. Among selachii elasmobranchs (sharks), three common species (*Carcharhinus limbatus*, *Carcharhinus leucas*, and *Sphyrna lewinii*) have been reported during our fish collection campaigns in San Juan, Rio Negro and Boca Grande estuaries. These three species plus the white tip reef shark, *Triaenodon obesus,* and the nurse shark, *Ginglymostoma cirratum*, have been previously listed as frequent along the islands of Coiba archipelago based on visual census and environmental DNA list provided by UNESCO in collaboration with COIBA AIP Scientific Station. From February to October of 2025, six young-of-the-year (YOY) individuals were tentatively identified as lemon shark (*Negaprion brevirostris*), a species non-reported in this area before, were collected in Boca Grande estuary (two individuals during our dry season sampling campaign (February) and four individuals during the wet season (August–October)). Taxonomic and genetic data analysis support that collected individuals corresponded to lemon sharks, *N. brevirostris*. To corroborate morphological identification, genetic identification of five individuals was conducted using PCR amplification and product sequencing of a mitochondrial gene segment, cytochrome oxidase I (COI) and of the non-coding mitochondrial segment (control region). Sequences from both DNA segments showed a 99.17% and 99.83% similarity with GenBank referenced sequences for *N. brevirostris*. For COI *N. brevirostris* sequences accession numbers were generated and deposited in GenBank: PX453691, PX453712, PZ043670, and PZ036798. These are the first sequences deposited in GenBank for the region and for Coiba National Park. This is also the first confirmed report of the presence of this species in waters of Coiba National Park and in any other of the islands of the Eastern Tropical Pacific marine corridor. Therefore, these are the first reported DNA sequences deposited on GenBank of this species for Panama's Pacific waters and for the corridor. This information will enable scientists to compare these sequences with reference data from other regions and along the Eastern Tropical Pacific allowing assessment of philopatric behavior and local and regional connectivity patterns. The results will also be essential for informing new management and monitoring strategies in Coiba National Park, including the designation of the Boca Grande estuary as a nursery area and the potential establishment of a shark sanctuary.

Keywords Eastern Tropical Pacific · Tropical estuaries · Carcharhinidae · Demographic connectivity · Nursery areas in sharks · Shark conservation

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Introduction

Estuarine fish fauna is highly diverse in the tropics. This elevated diversity supports a wider range of habitats, a larger species pool, stable environmental conditions, and accelerated evolutionary processes. In these productive blue carbon ecotones, predators are abundant and play a key role as indicators of ecosystem health and complex trophic dynamics (Blaber 2013). Shark species may be permanent or temporary estuarine residents, using these areas as nurseries and feeding grounds (Roundtree and Able 1996; Heupel et al. 2007). Along the Eastern Tropical Pacific, shark estuarine species are frequently reported, i.e., *Carcharhinus leucas*, *Carcharinus limbatus*, *Rhizoprionodon longurio*, *Mustelus lunulatus*, *Mustelus henlei*, and *Sphyrna lewinii* (Robles et al. 2015; Morales-Saldaña et al. 2025).

The lemon shark, *Negaprion brevirostris* (Poey, 1868), is a coastal tropical species distributed across the Eastern Pacific, Eastern Tropical Pacific, Western Atlantic, and Caribbean Sea (Del Moral-Flores et al. 2016). The species preferentially inhabit warm, shallow estuarine environments associated with mangroves and sandy substrates (DeAngelis et al. 2008; Ruiz-Abierno et al. 2020; Forero-Bastidas et al. 2023). Despite this habitat preference, *N. brevirostris* appears to be infrequently recorded in coastal and estuarine waters within regions where it is otherwise considered common, such as the Caribbean and Eastern Pacific (Forero-Bastidas et al. 2023).

Genetic information for the species in the Pacific is currently unknown and only available for Atlantic populations (Feldheim et al. 2001). However, phylogeographic and seascape genetic information indicate isolation of *N. brevirostris* Eastern Pacific populations after the emergence of the Isthmus of Panama (3.5 million years ago) (Schultz et al. 2008). Along the Eastern Tropical Pacific reports for the species and the synonymous *Negaprion fronto* (Jordan & Gilbert, 1882) are only present in coastal areas which do not include islands along the corridor (Froese and Pauly 2026).

Coiba National Park, the largest marine protected area in Central America, supports a high ichthyofaunal diversity, with 814 fish species recorded to date. However, estuarine, mangrove, and coral reef habitats within the park collectively harbor a more limited assemblage of 166 fish species (Vega and Villarreal 2003). Within Coiba National Park, estuarine, mangrove, and coral reef habitats collectively account for 166 recorded fish species (Vega and Villarreal 2003). Existing species inventories from Coiba's estuaries are largely restricted to major riverine systems—such as the San Juan, Río Amarillo, Río Negro, and Boca Grande, the largest rivers of the archipelago—and have primarily focused on teleost fishes (Nuñez et al. 2010). To date, published studies documenting shark assemblages in Coiba National Park and other insular regions of Panama have reported only *Carcharhinus limbatus*, *Carcharhinus leucas*, *Triaenodon obesus*, and *Ginglymostoma cirratum* (Vega and Villarreal 2003). *Negaprion brevirostris* has not been previously recorded in assessments of the fish fauna of Coiba National Park, and its occurrence in the waters of the largest marine national park and island in the Pacific of Central America has remained unconfirmed until now.

The year-round presence of young-of-the-year (YOY) individuals documented in this study provides strong evidence supporting the designation of specific estuaries within Coiba National Park, such as Boca Grande, as nursery areas for this species. Fish surveys conducted across Pacific Island systems off Panama do not identify *Negaprion brevirostris* as a common component of these assemblages, and the species has not been reported from the Coiba Archipelago or the Gulf of Chiriquí (Vega and Villarreal 2003; Nuñez et al. 2010). Although *N. brevirostris* is included in the most recent list of commercial fish species for the Republic of Panama, this compilation is based primarily on observational records and taxonomic information provided by local non-governmental organizations, without genetic confirmation of specimens or sampling from other major fishing regions, including the Gulf of Chiriquí, Gulf of San Miguel, and Gulf of Montijo (Garcés, 2021). Our novelty findings indicate that *Negaprion brevirostris* has not been previously recorded in Coiba National Park and does not constitute a common component of shark assemblages in Pacific waters of Panama. Consistent with this, the species is absent from records of the most abundant sharks in Panamanian Pacific fisheries, where approximately 83% of reported catches are dominated by other species, including, but not limited to, *Sphyrna lewini*, *Carcharhinus falciformis*, *Carcharhinus porosus*, *Mustelus lunulatus*, and *Alopias pelagicus* (Guzmán et al. 2020; Morales-Saldaña et al. 2025).

This short note presents the first unequivocal, documented record confirming the presence of *Negaprion brevirostris* in the waters of Coiba National Park. Species identification was supported by diagnostic morphological characteristics based on taxonomic keys applied to six young-of-the-year (YOY) individuals and further corroborated by genetic analyses of three of these specimens using two mitochondrial gene markers (COI and control region) (Ward et al. 2005; Tillett et al. 2012).

Material and methods

Sampling site and sample collection

Coiba National Park is the largest marine protected area in Central America encompassing 216,000 hectares of marine surface (ANAM 2009; Botero et al. 2024) (Fig. 1). This

Fig. 1 Boca Grande Estuary at Coiba National Park, Panama. Color dots indicate the studied transects for determining fish composition. All *N. brevirostris* were collected at T1 intermediate to high salinity 16 to 29 ppt

conservation area contains extensive important tropical marine ecosystems (coral reef, rocky shores, mangroves, mud platforms, sand beaches and estuaries) along its 339 km coastal line. Within this marine seascape, Coiba is the largest island covering an area of 503 Km^2. Eighteen rivers are registered on Coiba Island, from which the San Juan, Rio Negro, Barco Quebrado, Rio Amarillo and Boca Grande are the largest (ANAM 2009). Fish samples (bony and elasmobranchs) were collected across all river estuaries following a salinity gradient as part of a fish inventory and monitoring program that Coiba Scientific Station currently leads on estuary areas funded by the National Secretary of Science Technology and Innovation (SENACYT).

For shark collection we used a 50-m net trammel with 10-cm net opening commonly used in local artisanal fisheries. The net was deployed for a period of two to four hours and retrieved either at the end of this interval or immediately upon indication of a shark capture, as signaled by the submergence of a buoy. The trammel net was then hauled aboard, and the captured individual was manually transferred to the vessel and placed in a dissection tray; a small amount of muscle tissue was collected with a biopsy device (Fig. 2). The collected muscle tissue was placed in a 1.5-ml Eppendorf tube with 70% ethanol. Once the tissue was collected and preserved, animals were immediately released after observing a motility response. In cases where a motility response was not immediately observed, a controlled aquatic ventilation maneuver was applied to the shark before its release (Horton et al. 2023).

Fig. 2 Tissue biopsy for DNA conducted on N. brevirostris individuals

Tissue samples were obtained during both dry- and wet-season field campaigns conducted in the largest estuaries of Coiba Island National Park—San Juan, Río Negro, and Boca Grande. Muscle tissue biopsies were collected from three juvenile sharks captured in the Boca Grande estuary (Fig. 2).

Fig. 3 Young-of-the-year (YOY) of *N. brevirostris* collected in Boca Grande estuary, Coiba National Park

Molecular methods and genetic identification

DNA was extracted from tissues using DNA easy Blood and Tissue kit (QIAGEN, Inc., Valencia, CA, USA). DNA of extracted samples was checked for concentration and quality control using a nanodrop (Thermo Scientific, Waltham, MA, USA, https://www.thermofisher.com/pa/en/home.html accessed on September 2025). For COI a segment of 605 bp was amplified by PCR reactions using FISH universal primers (Ward et al. 2005). PCR reactions for this gene were performed under the following conditions: an initial denaturation for 1 min at 95 °C, follow by 5 cycles of denaturation at 95 °C for 30 s, hybridization at 50 °C for 40 s and extension at 72 °C for 1 min. Followed by 35 cycles of denaturation at 95 °C for 30 s, hybridization at 55 °C for 40 s with and an extension of 72 °C for 1 min. For control region gene segment, PCR reactions were conducted at the following conditions: an initial denaturation step of 94 °C for 1 min and 30 s followed by 35 cycles of a denaturation step at 94 °C for 10 s, an annealing step at 59 °C for 30 s followed by an extension step at 72 °C for 1 min and a final extension step of 5 min at 72 °C. Obtained sequences were aligned, edited and trimmed using Geneious Prime (Kearse et al. 2012). Afterwards sequences ID from COI and control region were corroborated using NCBI BLAST search, web version, with default settings (Altschul et al. 1990; Camacho et al. 2009). Also, the existence of previous sequences for this species through the region was also checked in the Barcode of Life (BOLD) (version4/taxonomy browser for species and species with sequences).

Taxonomic identification

Taxonomic identification was performed based on diagnostic morphological and meristic features described for the species (Compagno 1984b, 2002; Allen and Robertson 1994; Ebert et al. 2021). Specimens were characterized by a robust, fusiform body; short, broad snout; second dorsal fin slightly smaller but similar in shape to the first; and uniform yellow-brown coloration without distinctive markings (Fig. 3). This identification was further supported by morphometric comparisons with previously published juvenile datasets. YOY individuals captured in previous comparable nursery studies of the species where species size ranged from 58 to 157 cm total length (TL), while neonates are characterized by an open umbilical scar (Fig. 4) based on previously published size ranges for neonate and YOY of *N. brevirostris* (e.g., Freitas et al. 2006; Reyier et al. 2014; Ruiz-Abierno et al. 2020).

Results

Species-level identification

Morphological identification of six young-of-the-year (*N. brevirostris*) individuals—four females and two males collected in 2025 (February, August, and October) was conducted. Specimens ranged from 50.8 to 78.0 cm total length (mean = 68.54 cm) and weighed between 1.26 to 4.0 kg (mean = 2.21 kg). All individuals were captured in the Boca Grande estuary, specifically along the intermediate to high-salinity transect (Fig. 1). The taxonomic identity of all collected *Negaprion brevirostris* specimens was verified

Fig. 4 Ventral features of mouth, teeth and open umbilical scar of *N. brevirostris*

using the key diagnostic characters of Allen and Robertson (1994) and corroborated with the morphological descriptions provided by Compagno (1984a, b, 2002). As illustrated in Fig. 3, the first dorsal fin originates behind the rear margin of the pectoral fins, while the second dorsal fin is comparable in size or slightly smaller than the first dorsal fin (Compagno 1984a, b, 2002; Ebert et al. 2021) (Figs. 3 and 4). Ventral-view photographs of both individuals were also taken to document body profile, fin insertions, and other ventral characters that further support the identification of collected individuals as *N. brevirostris* (Fig. 4).

Genetic results

DNA from four out of six collected individuals confirmed species identity as *N. brevirostris*. Results showed more than 99.17% of similarity and 100% of query cover for referenced COI sequences for *N. brevirostris*. And 99.83% of similarity and 97% of query cover for referenced control region sequences of *N. brevirostris* (Schultz et al. 2008). One unique haplotype was obtained for COI sequences. Accession numbers were generated for all sequences: PX453691, PX453712, PZ043670, and PZ036798. Molecular information together with morphological, meristic information confirms the occurrence of *N. brevirostris* in waters of Coiba National Park.

Discussion

The identity of our specimens is consistent with published descriptions of the species (Cervigón et al. 1993; Compagno 1984a). Additional diagnostic characteristics found in the collected individuals are all in agreement with the descriptions by Compagno (2002) and Ebert et al. (2021).

Evidence of lemon sharks in Coiba National Park has important implications for the management and conservation of shark species within this major marine protected area. Thus, despite we sampled other estuarine areas of Coiba National Park (i.e., San Juan, and Rio Negro estuary) and always followed a salinity gradient during our sampling campaigns, all collected lemon sharks were only found at Boca Grande estuary first transect (intermediate to high salinities) close to the mouth of the estuary in areas fringed by red mangrove (*Rhizophora mangle*) (Fig. 1). Bottom features of this estuary area corresponded to sandy bottoms with similar physiographic characteristics documented by other researchers as suitable habitat for *N. brevirostris* (Forero-Bastidas et al. 2023).

The exclusive presence of YOY of *N. brevirostris* in the Boca Grande estuary throughout the year, and their absence from other estuarine systems of the Coiba National Park such as San Juan, Rio Negro, Barco Quebrado and Rio Amarillo, despite our sampling efforts during the year, suggests potential birthing site fidelity behavior in adult females. Philopatric behavior has been reported in other coastal and estuarine shark species (Hueter et al. 2004) including *N. brevirostris* (Chapman et al. 2009). For instance, Sundstrom et al. (2001) demonstrated through tagging studies, that adult female *N. brevirostris* exhibit philopatry in the Bahamas, returning to their birth nurseries to mate or give birth. Similarly, Feldheim et al. (2002) reported comparable findings using tagging and genotyping in adult females in the Bahamas.

Another important finding indicating the ecological significance of this estuary for sharks is that the collected *N. brevirostris* individuals shared their habitat with neonates and YOY individuals from other shark species, including the blacktip shark *Carcharhinus limbatus* and the bull shark *C. leucas* (Díaz-Ferguson et al. unpublished data). Also, the presence of juveniles of multiple species of sharks in the same estuary area potentially indicates habitat partitioning among these species (Simpfendorfer et al. 2005; DeAngelis et al. 2008). These observations support the designation of the Boca Grande estuary as a potential nursery area and, more broadly, as a candidate site for shark sanctuary status or as an Important Shark and Ray Area (ISRA). Rohner et al. (2025) emphasized that the quality and functionality of habitats, i.e., the presence of critical habitats that support life history functions, should be prioritized instead of over species range when developing effective shark conservation strategies. This research work also provides essential and pioneer information in terms of local, sub regional and regional connectivity patterns, natal site fidelity and conservation of *N. brevirostris*.

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