UNIVERSIDAD DE COSTA RICA

SISTEMA DE ESTUDIOS DE POSGRADO

DISTRIBUCIÓN DEL PEZ SIERRA DE DIENTES GRANDES (*Pristis pristis*) EN COSTA RICA: RECONSTRUCCIÓN HISTÓRICA Y ACTUAL MEDIANTE MÉTODOS TRADICIONALES Y ADN AMBIENTAL

Tesis sometida a la consideración de la Comisión del Programa de Estudios de Posgrado en Biología para optar al grado y título de Maestría Académica en Biología

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Ciudad Universitaria Rodrigo Facio, Costa Rica

2021

Dedicatoria

A la memoria de Walter Eduarte

"La muerte es un matiz de la existencia
morir es florecer en otra forma
la caduca materia se transforma
en ser nuevo, en rosales o en esencia

Allá en el camposanto
que esmaltan las auroras de amaranto
y las tardes de sándalo y carmín,
allá donde la hiedra
abraza con amor la cruz de piedra,
anhelo ahora descansar al fin"

Lisímaco Chavarría

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“*To withstand tragedy and malevolence without becoming corrupt*”
J.B. Peterson

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Índice

LISTA DE CUADROS

LISTA DE FIGURAS

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INTRODUCCIÓN

Debido a la gran velocidad a la que muchas especies están desapareciendo, es importante entender cómo se distribuyen espacial y temporalmente, así como el conjunto de amenazas que afectan a sus poblaciones (La Marca et al. 2005; McCallum et al. 2007; Brook et al. 2014; Harrison & Dulvy 2014; Ceballos et al. 2015; Lusardi et al. 2015; Dulvy et al. 2016). En décadas recientes, las especies de tiburones y rayas (elasmobranquios) han sufrido reducciones considerables en el tamaño de sus poblaciones, principalmente debido a la sobrepesca (Stevens et al. 2000; Baum et al. 2005; Dulvy et al. 2008; Ferretti et al. 2010). De acuerdo a una evaluación global de la Unión Internacional para la Conservación de la Naturaleza (UICN), un 25% de todas las especies de elasmobranquios se encuentran en riesgo de extinción, y este número podría ser aún mayor, ya que para casi un 50% de las especies no hay información suficiente para evaluar el estado de sus poblaciones (Dulvy et al., 2014).

Uno de los grupos de elasmobranquios más amenazados es el de los peces sierra, pertenecientes a la familia Pristidae (Dulvy et al. 2016). Las cinco especies de peces sierra están en riesgo de extinción según la UICN, lo que significa que han experimentado reducciones poblacionales importantes, algunas de hasta un 95% en los últimos 50 años (Carlson et al. 2013; Dulvy et al. 2016). Por lo tanto, generar información sobre los patrones de distribución espacio-temporal, así como identificar las principales amenazas que afectan a los peces sierra es crucial para desarrollar medidas de manejo y conservación que permitan la recuperación de sus poblaciones (Harrison & Dulvy 2014).

El pez sierra de dientes grandes (*Pristis pristis*) es la especie del grupo que se distribuye más ampliamente, presentando una distribución circumtropical (Faria et al. 2013). Globalmente, la especie está distribuida en cuatro unidades geográficas o subpoblaciones que incluyen el Atlántico Oriental, el Atlántico Occidental, el Pacífico Oriental y el Indo-Pacífico Occidental (Faria et al. 2013). Sin embargo, se estima que *P. pristis* ha sufrido una reducción de hasta el 61% en su ámbito de ocurrencia, por lo que ha actualmente la especie se encuentra en peligro crítico de extinción según la UICN (Kyne et al. 2013).

La pesca ha sido identificada como la principal causa de reducción poblacional y riesgo de extinción de *P. pristis* a nivel mundial (Kyne et al. 2013). Dadas sus características de vida, incluyendo madurez tardía (8-10 años), baja fecundidad y bajas tasas de crecimiento poblacional (0.03- 0.07 año^{-1}), esta especie es altamente vulnerable a la presión pesquera, inclusive a bajas tasas de captura (Thorson 1976; Simpfendorfer 2000; Peverell 2009). La vulnerabilidad de la especie también radica en que al ser de gran tamaño (>6.5m) y poseer un rostro en forma de sierra, se enreda fácilmente en redes y otras artes de pesca, por lo que las pesquerías a nivel costero, como trasmallos y arrastre de fondo, han jugado un papel importante en la reducción de sus poblaciones (Simpfendorfer 2000; Dulvy et al. 2014; Harrison & Dulvy 2014).

La destrucción y degradación del hábitat también ha contribuido de forma sustancial a la reducción de las poblaciones de *P. pristis* (Kyne et al. 2013; Dulvy et al. 2016)*. Pristis pristis* habita ríos, lagos y ecosistemas costeras poco profundos (<100 m), como playas arenosas y fangosas, pastos marinos y lagunas. (Thorson 1976; Thorburn et al. 2007; Peverell 2009; Harrison & Dulvy 2014). Los juveniles de la especie dependen de manglares y ríos durante los primeros años de vida (Thorburn et al. 2007; Peverell 2009; Whitty et al. 2017). Los manglares son severamente afectados por diversas actividades antropogénicas como la agricultura, acuicultura y el desarrollo costero (Polidoro et al. 2010; Norton et al. 2012), como resultado la cobertura de manglar ha disminuido globalmente entre 20 y 35 % entre 1980 y 2010 (Valiela et al. 2001; FAO 2007; Polidoro et al. 2010). Además, los ríos en los que habita *P. pristis* son afectados por represas y otras obstrucciones de los cauces, contaminación, sedimentación y dragado (CITES 2007; Thorburn et al. 2007).

Latinoamérica es parte del área geográfica en la que se distribuyen las subpoblaciones de *P. pristis* del Atlántico Occidental (AO) y Pacífico Oriental (PO). En esta región, los peces sierra han habitado una gran variedad de ambientes acuáticos, incluyendo ríos, humedales, manglares, estuarios y aguas costeras entre México y Uruguay (AO) o entre México y Perú (PO), aunque los registros anecdóticos menores a 10 años, provienen de Perú, Colombia, Panamá, Nicaragua y Costa Rica (Cook et al. 2005; Faria et al. 2013; Mendoza et al. 2017). En Costa Rica, los estudios científicos enfocados en *P*. *pristis* se han realizado en la comunidad de Barra del Colorado sobre el río Colorado, que forma parte del Sistema Lago de Nicaragua (Thorson 1976, 1982a, 1982b). Además, existen registros históricos de la especie en diversos ecosistemas costeros y de agua dulce. El registro más antiguo data del año 1859 en el río Sarapiquí (Hilje & Fournier 2017), aunque ha sido un habitante común de río Frío, al que la cultura indígena Maleku en la zona norte del país ha conocido históricamente (A. Acosta Blanco, miembro de comunidad Maleku, *com. pers.,* 2016). Esta especie también ha sido registrada en otros tributarios del río San Juan y en ecosistemas del Pacífico Norte, Central y Sur de Costa Rica (Winemiller 1983, Chicas-Batres 1995; Bussing 1998), aunque los registros científicos más recientes datan de la década de 1990 por Chicas-Batres (1995) en el Pacífico Sur del país en el Humedal Nacional Térraba-Sierpe.

Diversas técnicas se han utilizado para el estudio de *P. pristis* a nivel mundial, principalmente métodos visuales y de pesca, aunque en ocasiones resultan poco eficientes debido a los hábitos crípticos de la especie y a su baja abundancia al estar en peligro crítico de extinción (Simpfendorfer et al. 2016). Los muestreos visuales son poco efectivos debido a su naturaleza bentónica y preferencia por aguas turbias (Simpfendorfer et al. 2016; Cooper et al. 2021). Los métodos tradicionales de pesca como líneas y trasmallos han sido exitosos en el estudio de especies de peces sierra, aunque pueden fallar aun estando presente la especie. Además, algunos métodos de muestreo son costosos y difíciles de emplear en áreas remotas (Thorburn et al. 2007; Wiley & Simpfendorfer 2010). Por otro lado, el uso de entrevistas ha resultado exitoso en la identificación de hábitats importantes y para examinar cambios en la distribución y abundancia de peces sierra a través del tiempo (Hossain et al., 2014; Leeney, 2017; Leeney & Downing, 2016; Leeney & Poncelet, 2015; Poulakis & Seitz, 2004). Sin embargo, el método está limitado al alcance del proyecto, y a la voluntad de las personas a contribuir con el reporte de captura de una especie protegida (Simpfendorfer et al. 2016).

El ADN ambiental (eDNA) es una técnica novedosa, capaz de detectar la presencia de especies terrestres y acuáticas mediante trazas de ADN presentes en el ambiente que habitan (Rees et al. 2014). El eDNA tiene mayor capacidad de detección de especies crípticas o cuyas poblaciones se han reducido considerablemente comparada con otros métodos tradicionales (Thomsen et al. 2012; Valentini et al. 2016; Wilcox et al. 2016). Las posibles trazas de ADN a detectar provienen de lisis celular o de excreciones y/o secreciones (heces, saliva, orina y células epiteliales) de la especie o especies de interés (Dejean et al. 2011). Este ADN puede permanecer viable desde horas hasta miles de años según las condiciones ambientales (Thomsen et al. 2012), aunque en ambientes acuáticos, la degradación del eDNA ocurre en cuestión de días o semanas (Thomsen et al. 2012, 2016). Esta técnica ya ha sido validada y utilizada con éxito para detectar la presencia de *P. pristis* en ecosistemas tropicales dulceacuícolas y estuarinos de Australia (Simpfendorfer et al. 2016; Cooper et al. 2021). Debido a que la detección del eDNA en el medio es contemporánea con la presencia de la especie, su aplicación ayudaría a determinar la distribución actual de *P. pristis* en Costa Rica.

En nuestro país se realiza una fuerte y creciente actividad pesquera desde hace décadas, siendo el trasmallo, la línea de pesca y las redes de arrastre, los principales métodos utilizados en los ambientes costeros (Bussing & López 1994; Chacón et al 2007; Fournier & Fonseca 2007). En estos ambientes, la distribución y abundancia de especies de elasmobranquios es afectada por la presión pesquera (Chacón et al. 2007; Clarke et al. 2016). Los manglares son hábitats críticos para especies de elasmobranquios como *P. pristis* (Kyne et al. 2013), así que el incremento en la actividad pesquera en estos ecosistemas puede tener efectos negativos sobre sus poblaciones.

Debido a la gran reducción de las poblaciones de *P*. *pristis*, y a que en Latinoamérica la especie posiblemente esté restringida a ríos y otros humedales en zonas remotas de Centro América, determinar su distribución actual y hábitats esenciales para su sobrevivencia en Costa Rica es fundamental. Esta información también será vital para identificar áreas dentro de su distribución en donde se deben enfocar futuros esfuerzos de conservación, lo cual podría permitir la recuperación de sus poblaciones (Simpfendorfer et al. 2016). La distribución y estado actual de las poblaciones de *P. pristis* en Costa Rica es incierta, ya que está limitada a la poca información reportada por pescadores. La información de entrevistas a pescadores del Golfo Dulce en el 2004 (Kyne et al. 2013) y la antigüedad de los registros científicos de *P. pristis* en Costa Rica, sugieren una reducción de sus poblaciones en el país. Ya que varios estudios han evidenciado reducciones poblacionales y extinciones locales de *P. pristis* (Kyne et al. 2013; Dulvy et al. 2016), este estudio pretende determinar los cambios en la distribución actual e histórica del pez sierra de dientes grandes en Costa Rica. Además, es necesario identificar hábitats críticos para la especie, así como las principales amenazas que enfrenta en las diferentes regiones para generar medidas de conservación efectivas orientadas a la recuperación de sus poblaciones. Determinar el estado actual de las poblaciones de *P. pristis* en Costa Rica ayudará a solventar los vacíos de información sobre la especie a nivel regional, colaborando así con una evaluación global completa.

CAPÍTULO 1

A beacon of hope: distribution and current status of the largetooth sawfish in Costa Rica

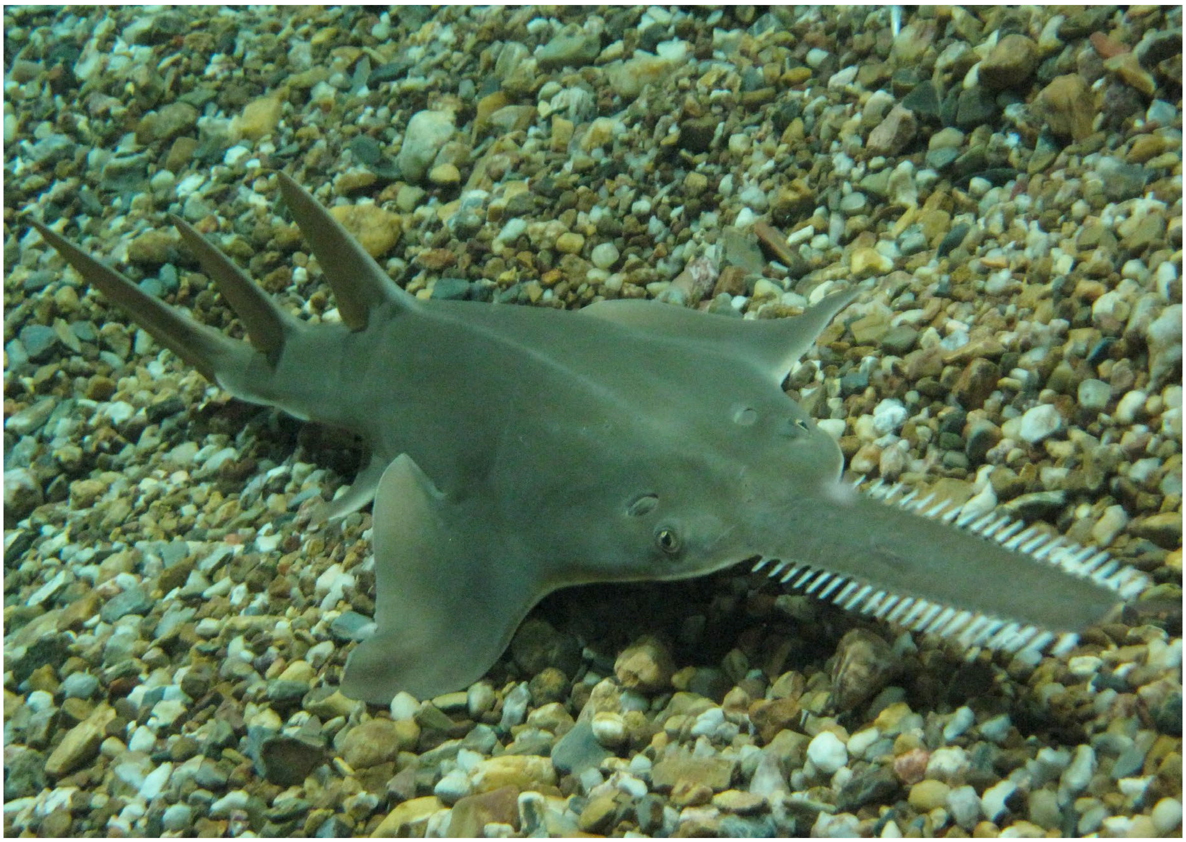

Plate 1 Photo of a Largetooth sawfish in a riverine environment. Photo credit: Kate Buckley.

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ABSTRACT

The Critically Endangered largetooth sawfish *Pristis pristis* is one of the most threatened elasmobranch species and is currently thought to be locally extinct in at least 27 countries. Although largetooth sawfish information in Central America is scarce, recent records show that this species is still present in Costa Rica, yet its distribution and current status remain unclear. This study investigated the spatial and temporal distribution of the largetooth sawfish in Costa Rica and identified local threats affecting the populations. We conducted 275 structured interviews in coastal and riverine communities across the country, which resulted in 134 confirmed records in the Pacific, 1 in the Caribbean and 51 in the northern region. Historical and recent records suggest the largetooth sawfish has undergone significant reductions in abundance and distribution from coastal and riverine areas, mainly due to interaction with fishing gear such as gill nets and hook and line. Most sawfish captured by gill nets were reported in the Central Pacific region, whereas hook and line records were more common in the northern region and the South Pacific. Although largetooth sawfish populations in Costa Rica have followed the global decline trend, we found 2 main hotspots where recent sightings and captures appear to be more common, suggesting there is still hope for the species to recover in Costa Rica and possibly in the region. Moreover, Costa Rica recently became the 17th country to ratify national legal protection for sawfishes, which may strengthen conservation efforts to protect populations locally and in the Central American region.

KEY WORDS

Pristis pristis; maps; hotspots; Central America; legal protection; interviews

1. INTRODUCTION

Sawfishes (Pristidae) are the most threatened group of cartilaginous fishes (Dulvy et al. 2016), with all 5 species listed as either Critically Endangered or Endangered by the International Union for the Conservation of Nature (IUCN) Red List (Harrison & Dulvy 2014). Throughout their distribution, sawfishes have experienced significant population declines (>95%) and reductions in geographic range of up to 81%, which has resulted in extirpation and/or local extinctions (Carlson et al. 2013, Dulvy et al. 2014). The Critically Endangered largetooth sawfish *Pristis pristis*, once a circumtropical species, has become locally extinct in 27 countries, and the lack of data from Central America has hindered management and conservation efforts to ensure its long-term survival in the region (Dulvy et al. 2014, Mendoza et al. 2017). Sightings in Nicaragua, Panama and Costa Rica over the past 10 yr suggest that the species may still be present in Central America, but its current distribution and status remain unclear.

Coastal fisheries, including bottom trawling and gillnets, have played a major role in the decline of the largetooth sawfish throughout its distribution (Kyne et al. 2013, Harrison & Dulvy 2014). Fishing, even at low levels of exploitation, has been identified as the main driver of the global decline and extinction risk of the largetooth sawfish (Kyne et al. 2013). Given their slow growth rates, late maturity (8 to 10 yr), and low fecundity (7.3 mean litter size), largetooth sawfish have extremely low intrinsic rates of population increase (0.03 to 0.07 yr^{-1}) (Thorson 1976, Simpfendorfer 2000, Peverell 2009). Moreover, this species is characterized by its large size (>6.5 m total length), slow-moving behavior and distinctive long toothed rostrum, making it extremely vulnerable to entanglement in nets and other fishing gear.

Substantial habitat loss and degradation of critical habitats has also contributed to the decline of largetooth sawfish populations (Peverell 2009, Hossain et al. 2015). As a coastal euryhaline species, the largetooth sawfish occurs in a wide variety of coastal and riverine habitats. Adults are mainly found in marine and estuarine environments, while juveniles have greater affinity for rivers, mangroves and wetlands (Kyne et al. 2013), all of which are substantially impacted by human activities (e.g. agriculture, aquaculture, and dams) and/or rapid coastal or riverine development (CITES 2007, Peverell 2009, Hossain et al. 2015). The largetooth sawfish is also vulnerable to climate change, as predicted changes related to sea level rise and rainfall or runoff regimes could affect the structure and function of their critical coastal and estuarine habitats (i.e. mangroves, rivers and wetlands) (Chin et al. 2010, Polidoro et al. 2010, Wenger et al. 2011, Kyne et al. 2013).

The largetooth sawfish has a global tropical or subtropical distribution consisting of 4 subpopulations: Eastern Atlantic, Western Atlantic (WA), Eastern Pacific (EP) and Indo-West Pacific (Faria et al. 2013, Dulvy et al. 2016). The WA and EP subpopulations occurred in coastal and freshwater ecosystems of countries in Latin America (Cook et al. 2005, Faria et al. 2013). However, according to recent studies, populations of largetooth sawfish have almost disappeared or are extirpated from México (Bonfil et al. 2017), Peru (Mendoza et al. 2017), Colombia and Venezuela (Gómez-Rodríguez et al. 2014). In some countries of Central America, knowledge about its current distribution remains uncertain due to the lack of studies and/or recent sightings (Dulvy et al. 2014). In Central America, the WA subpopulation presents scattered reports from Panama, Nicaragua, Honduras and Belize, while in the EP, recent records are only available from Nicaragua and Panama, and the species has been declared locally extinct from Guatemala (Kyne et al. 2013).

Historically, the largetooth sawfish inhabited the Pacific and Caribbean coasts as well as the northern region of Costa Rica. However, most of the information available on the species is from research expeditions conducted in the 1970s and 1980s and is restricted to a few locations in the northern region (San Juan-Colorado river) and the Caribbean (Thorson 1976, 1982a,b). The species has also been reported in the North, Central and South Pacific regions (Winemiller 1983, Chicas-Batres 1995, Bussing 1998), with additional sightings in some of the main tributaries of the San Juan River (Bussing 1998), including a report from the Sarapiquí River by a Swiss settler in 1869 (Hilje & Fournier 2017). Although some interviews in the South Pacific region from 2004 and 2011 suggest that the Corcovado National Park and Térraba- Sierpe National Wetlands (TSNW) may still hold viable populations, fishers from Gulf Dulce (South Pacific region) claim they have not seen a sawfish in decades (Kyne et al. 2013).

Given recent global population declines and local extinctions of largetooth sawfish, knowledge of their distribution, abundance and threats impacting their populations, particularly in data-poor countries, remains crucial to developing effective management and conservation approaches at both local and regional scales. This study investigated the status of the Critically Endangered largetooth sawfish in Costa Rica by collecting local ecological knowledge through interview data in coastal and riverine communities. Our study addressed an important challenge previously identified in the literature, which was generating detailed data on the freshwater distribution of the largetooth sawfish (Dulvy et al. 2016, Fernandez-Carvalho et al. 2014). Moreover, we provided critical information on local threats affecting sawfish in the entire country. This information is necessary to develop effective conservation measures for the species in the Eastern Pacific (Kyne et al. 2013). Specifically, we (1) determined the current and historical distribution of the species in the entire country; (2) identified local threats affecting their survival; and (3) gathered information about the cultural importance and uses of the species to locals. These outcomes provide a more comprehensive understanding of the current status of largetooth sawfish in Costa Rica and contribute to a complete global evaluation of the species. In addition, we documented some records of the smalltooth sawfish *Pristis pectinata* in the Caribbean of Costa Rica.

2. MATERIALS AND METHODS

2.1. Study area

The study was conducted in coastal and riverine communities from the Pacific, Caribbean and northern region of Costa Rica. The Costa Rican Pacific coastline is 1254 km in length and is characterized by numerous bays, gulfs, estuaries and mangrove forests (Cortés & Wehrtmann 2009). Mangrove cover is predominantly distributed near the mouth of large rivers, including the Tempisque (Gulf of Nicoya in the Central Pacific), and the Grande de Térraba and Sierpe (South Pacific). The lowlands of the latter 2 rivers form the TSNW, a highly productive, diverse and economically important wetland in Costa Rica (Mainardi 1996). The Caribbean coastline is smaller (212 km in length), less irregular and characterized by a small number of mangrove patches located mainly in the south (Gandoca-Manzanillo). In the central and north Caribbean, there are coastal lagoons associated with important rivers such as the Tortuguero, Matina, Pacuare and San Juan-Colorado (FAO 2004). The northern region of Costa Rica is characterized by numerous rivers and lagoon systems such as Caño Negro, one of the most important wildlife refuges in this region. All of the freshwater bodies in the region, including large rivers such as Sarapiquí, San Carlos and Frío, are major tributaries of the San Juan-Colorado River system (a natural boundary between Costa Rica and Nicaragua), which flows into the Caribbean Sea (Barrientos & Chaves 2008).

2.2 Interview surveys

Given that sawfish are rare and often restricted to poorly accessible areas, determining their current status and distribution in most places is challenging and has limited the implementation of effective conservation strategies (Simpfendorfer et al. 2016). Interviews have been employed as a valid ecological tool for evaluating sawfish distribution, although they are dependent on the willingness of interviewees to collaborate (Poulakis & Seitz 2004, Leeney & Downing 2016, Simpfendorfer et al. 2016). During this study, most interviewees were willing to collaborate and expanded on the answers requested, providing valuable information on the current and historical distribution of the largetooth sawfish *Pristis pristis* in Costa Rica. To determine the distribution and local threats affecting sawfishes in Costa Rica, we conducted interviews at 42 sites across the entire country (Fig. 1). Interviews were conducted by teams of 2 people after a training period. Structured interviews aimed to target older or more experienced fishers who could have more information on historical or recent sightings, captures and local threats that may have affected the populations of largetooth sawfish. Interviews were conducted at harbors, marine supply shops, bait shops, local fish markets, restaurants, schools, fisheries cooperative buildings or at the residence of the interviewee. Although interviewees were selected as they appeared, experienced fishers were the main target. After completing the interview, interviewees were asked to provide information about other people who knew or had captured the species. Interviewers were introduced as researchers from a Sawfish Conservation initiative at the University of Costa Rica, interested in documenting the species occurrence and identifying local threats. Interviewees were shown several pictures of the largetooth sawfish and asked for permission to conduct the interview before starting. A standard questionnaire was developed, based on a survey conducted by Conservation International and the Talking Oceans Foundation in Colombia. The questionnaire was adapted to include a series of questions from the Florida Program for Shark Research of the Florida Museum of Natural History. The questionnaire was applied to all interviewees.

Through the interviews, we compiled a database with historical and recent anecdotal records of sawfish sightings and/or captures for the entire country. These interviews aimed to gather detailed information on the most recent encounters by interviewees, including the date, location (approximate areas within Costa Rica), type of encounter (fished or sighted), capture method (gill net, seine, long-line, etc.), status of the animal (dead or alive), and uses (meat consumption, trophy, or decoration, etc.). In addition, we documented interviewees' perceptions of the causes of sawfish decline, why sawfish were once a relatively common species in some areas of Costa Rica but now seem rare or absent, and how local threats have impacted sawfish populations.

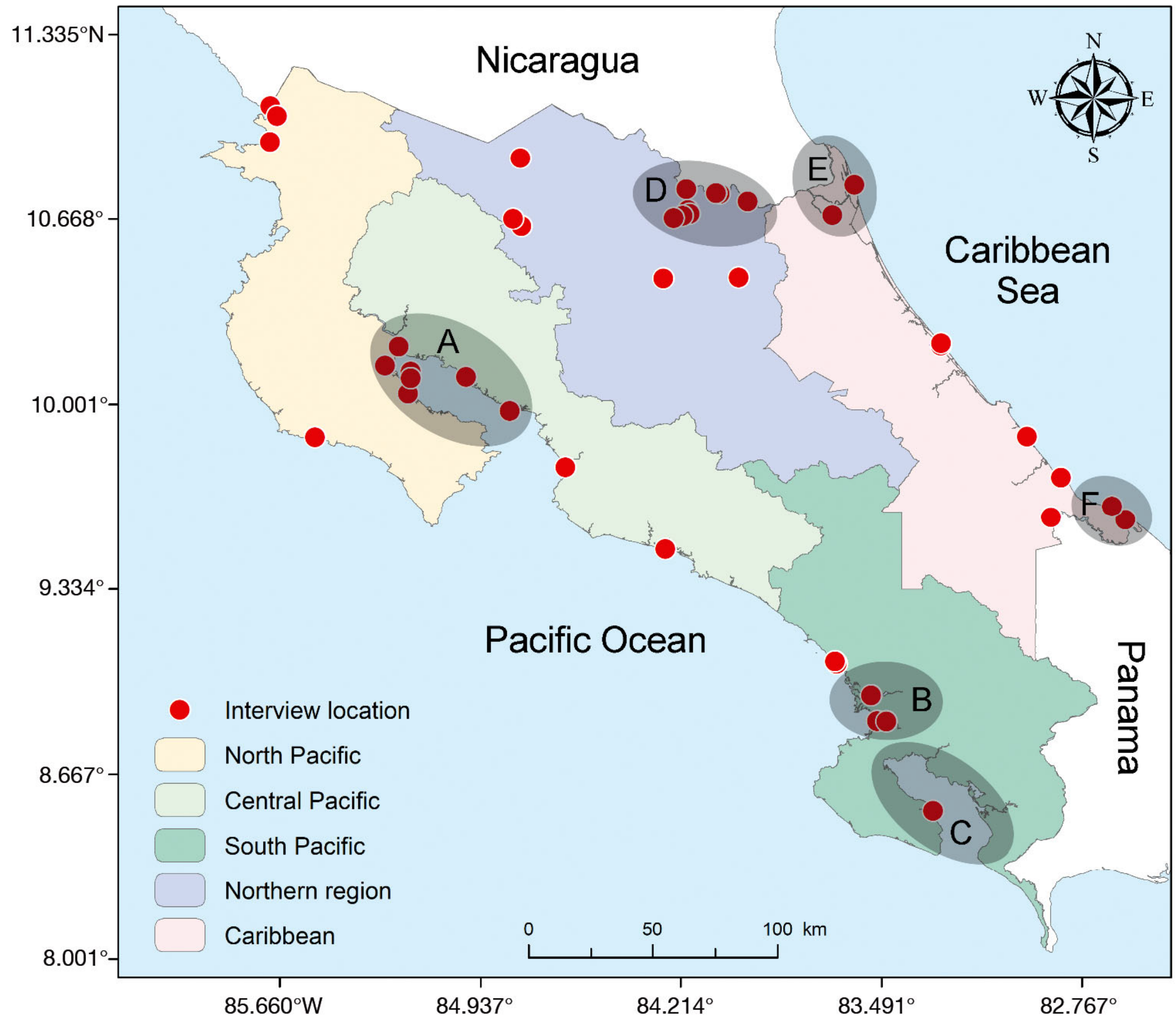

Figure 1. Interview locations in Costa Rica, Central America. (A) Gulf of Nicoya; (B) Térraba-Sierpe National Wetlands; (C) Gulf Dulce; (D) Boca San Carlos − Boca Cureña; (E) Barra del Colorado; (F) Gandoca, Manzanillo and Sixaola.

Selection of fishing villages to conduct interviews was based on historical records and proximity to suitable sawfish habitats, such as mangroves, large rivers and other key coastal habitats such as those mentioned by Thorson (1976, 1982a,b), Winemiller (1983), Chicas-Batres (1995), and Bussing (1998). Interviews were also conducted in other locations of the country when an interviewee who had had an encounter with a sawfish did not live in a coastal or riverine community. We conducted a total of 275 structured interviews from March 2016 to October 2017, covering a large number of coastal and riverine communities from Costa Rica (Table 1). Interviewees were categorized in the following age groups: <30, 30−45, 46−60 and >60 yr. Most interviewees (n = 88) were in the category >60 yr old, while 33 were <30 yr old. Out of 275 interviewees, 50 did not practice any type of fishing; 18 were sport fishers, 98 were commercial fishers (trawl, gill net, or longline) and 109 were subsistence or artisanal fishers (hook and line). From the 275 interviews, 259 interviewees knew the species in several ways: 15 had only seen it on TV; 36 knew it from stories, pictures or had seen dried rostra as decoration. In total, 208 (80%) of these 259 interviewees had had at least one encounter with a sawfish.

Table 1. Total number of interviews (n = 275) by region in Costa Rica, Central America.

Regions	No. of interviews
Pacific	
Northern Pacific	24
Nicoya Peninsula	7
Gulf of Nicoya	94
Central Pacific	2
Southern Pacific	53
Northern region	75
Central Valley	4
Caribbean	
Northern Caribbean	3
Central Caribbean	3
Southern Caribbean	10
Total	275

2.3. Species identification

Although our questionnaire aimed to gather general information on sawfishes, we were able to classify most records at the species level based on the species distribution and/or photographic records. Records from the Pacific were all assigned to the largetooth sawfish *P. pristis* (*P. pectinata*'s distribution is restricted to the Atlantic; Faria et al. 2013). As for the Atlantic (i.e. Caribbean coast), each record was assigned to either *Pristis* sp., *P. pristis* or *P. pectinata* depending on the quality of the evidence provided by each interviewee and any other additional evidence available.

2.4. Data analysis

To examine the spatial distribution of historical and recent sawfish sightings or captures in Costa Rica, we used the most accurate location of the last encounter of each interviewee from the database. Spatial information was categorized by region (North Pacific, Gulf of Nicoya, Central Pacific, South Pacific, Northern Region, and Caribbean), and habitat type (river, brackish or estuarine, lagoon, inshore and offshore). Sawfish records were also classified in the following time periods: >20, 11−20, 5−10, and <5 yr ago, using January 2018 as a reference date. Spatial data was plotted and the distribution mapped based on the time periods using ArcMAP 10.2® (ESRI 2014). These maps provided important information on (1) historical and recent changes in the distribution and abundance of largetooth sawfish in the entire country and (2) potential hotspots or critical habitats that may be important for the conservation of this species in Costa Rica.

In addition to reports from local interviews, we included 20 records from people who provided recent pictures or information that could validate sawfish records. Given that these records were not gathered through interviews, they were only used to map sawfish distribution and were not included in the interpretations of population trends as they could bias the analysis. Of the total records, 7 of the encounters took place in Nicaragua, and the remaining 222 records were within Costa Rican waters.

To assess if sawfishes were more frequently sighted or fished, and which fishing gear was responsible for catching them, information was summarized by type of encounter and fishing gear. Information on the use of sawfish products (meat, rostra and rostral teeth), people's perception towards changes in sawfish abundance through time and local threats driving sawfish decline or disappearance from Costa Rican waters were also analyzed.

3. RESULTS

There were 206 confirmed records of the largetooth sawfish *Pristis pristis* across the entire country and only 2 records of the smalltooth sawfish *P. pectinata* in brackish waters from the Caribbean. Fourteen additional records could not be classified to species level. Given that most confirmed records (93%) corresponded to the largetooth sawfish, major findings presented in the following sections are based exclusively on this species, hereafter referred to as sawfish.

Interview data revealed that sawfish have experienced a substantial decline through time (Fig. 2A). The number of sightings or captures declined from 89 historical records (>20 yr) to 24 in the past 5 yr; 8 interviewees did not specify a time period. Species' records decreased by 92% (Central Pacific), 80% (North Pacific), 54.5% (northern region) and 25% (South Pacific) (Table 2). Even though sawfish records from the South Pacific and northern region have decreased historically, records in both regions remained stable when comparing the 11−20 yr and <5 yr categories. Additionally, sawfish records from the northern region remained stable in the 3 most recent time categories (Table 2, Fig. 3).

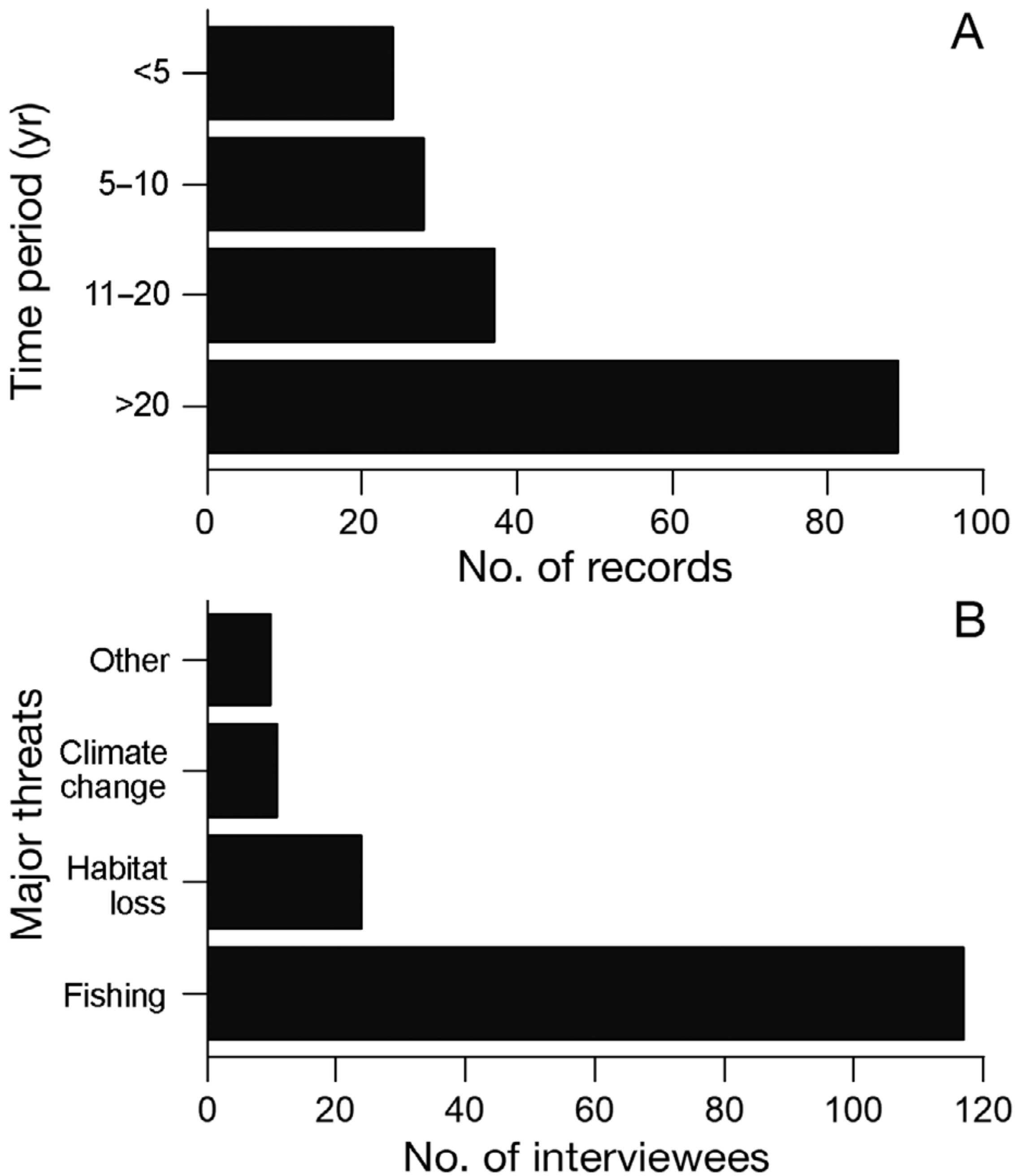

Figure 2. (A) Interview records (n = 178) for number of sightings of largetooth sawfish *Pristis pristis* through time in Costa Rica, Central America. Eight interviewees did not specify a time period for their encounter and are not included. (B) Sawfish threats mentioned by interviews; only answers from persons who had had an encounter in Costa Rican waters were included.

Table 2. Interview records (n = 178) for number of past sightings of largetooth sawfish *Pristis pristis* and additional reports (n = 20) in major regions of Costa Rica (Central America) by time periods, taking January 2018 as the reference date. Eight interviewees did not specify a time period for their encounter, so they are not included in this table. TSNW: Térraba-Sierpe National Wetlands.

Region	Time periods							
	>20 yr		11–20 yr		5–10 yr		<5 yr	
	Interviews	Additional reports	Interviews	Additional reports	Interviews	Additional reports	Interviews	Additional reports
North Pacific	5		4		2		1	
Central Pacific								
Nicoya Península	2				2			
Gulf of Nicoya	41		10		6		3	1
Central Pacific	7	2	1		1		1	1
South Pacific								
TSNW	9		8		4	1	5	6
South Pacific	1		2		3		3	
Dulce Gulf	2		2				1	
Northern region	22	1	9		10		10	7
Caribbean	0		1		0		0	1
Total	89	3	37	0	28	1	24	16

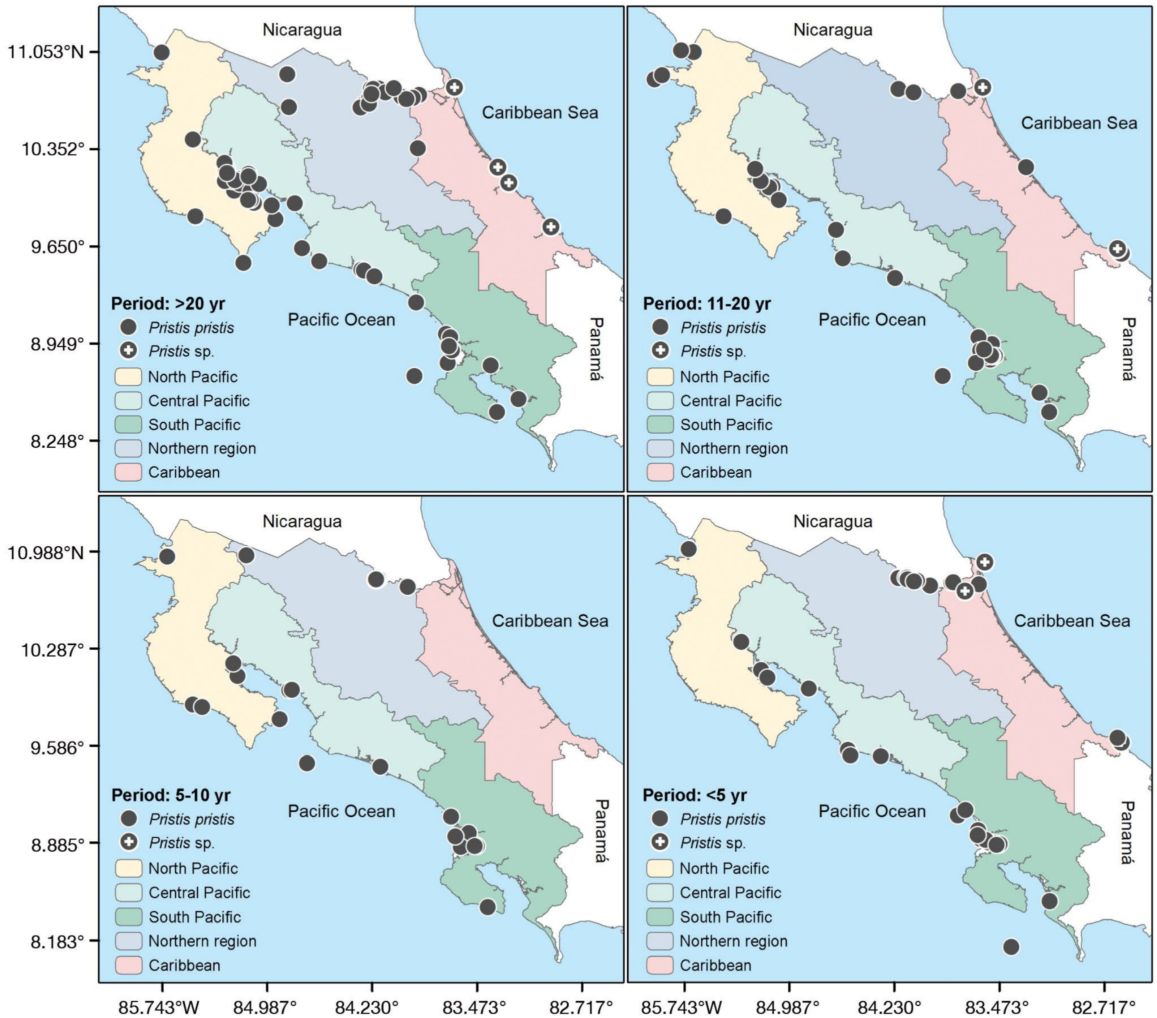

Figure 3. Estimated historical and current distribution of the largetooth sawfish *Pristis pristis* (●) in Costa Rica (Central America) from interview data (n = 186) and additional records (n = 20), taking January 2018 as the reference date. Eight interviewees did not give information on time period; therefore, those records were not included in the analysis. Fourteen sawfish *Pristis* sp. records (✪) in which the species could not be identified are included.

Sawfish records varied by region, with most sightings or captures recorded in the Central Pacific (41%), northern region (29%) and South Pacific (23%) regions. Locally, most records occurred in the Gulf of Nicoya (32%), San Juan River (21%) and TSNW (16%). Sawfish were sighted or captured more frequently in rivers (35%), brackish waters (34%) and inshore habitats (17%), while there were fewer records from offshore sites (7%) and lagoons (3%). Eight interviewees did not specify the habitat at the time of the encounter.

Based on our interviews and additional data, hook and line (32%) and gill net (31%) were responsible for capturing most sawfishes (Table 3). The remaining sawfish encounters were mainly sighted or harpooned, the latter was found to be a common practice in the northern region in recent years. Ten other sawfish were poisoned by an unknown chemical in the San Juan River (northern region); poisoned or suffocated after a massive molasses spill in the San Carlos River (northern region); entangled in barbwire, roots and tree branches; or found dead at the beach. Nineteen persons did not give information about fishing gear. Most sawfish captured in the northern region and South Pacific regions were by hook and line, whereas in the Central Pacific region, most sawfish were caught in inshore and brackish waters with gill nets (Table 3).

Of the 206 records, most sawfish (66%) were reported dead at the time or as a result of the encounter, whereas 24% were reported alive or the person did not give any information about the fate of the animal. Interview data regarding deceased sawfish indicate they were sold in local markets or used for consumption; 6 interviewees discarded the sawfish. A total of 12 interviewees reported cutting the saw at the time of the encounter; 2 of these interviewees were retired shrimp trawl captains who discarded the saw-less sawfish while it was still alive.

In terms of sawfish uses, 200 interviewees said that sawfish rostra were kept and used as trophies. Fishers from the Gulf of Nicoya mentioned that they historically (>20 yr) used to keep rostra for national customers who had previously ordered them. However, our interview results indicate that this was also a recent practice in the northern region, as several interviewees reported a Panamanian buyer regularly visited the area in search of rostra to use as decoration in a hotel in his home country. Traditional medicinal uses of sawfish rostra were only mentioned by 7 interviewees. Medicinal properties, such as curing headaches and prevention of baldness, did not involve the consumption of the rostrum; instead, the rostrum was used to comb one's hair to cure the illness. Another medicinal property was the extraction of calcium to make a supplement used for bone strengthening, but the process was not known by the interviewee. Additional uses for the rostral teeth were also reported, including making arts and crafts, fabrication of cock-fighting spurs, and to prepare invigorating beverages for pregnant women by grinding the sawfish teeth to a fine powder.

A large proportion (72%) of interviewees who had had an encounter with a sawfish mentioned that overfishing was the major threat responsible for sawfish decline. Other threats such as habitat loss or degradation (15%) and climate-related changes (7%) were also considered important. Ten interviewees mentioned that other causes such as emigration (n = 3), low reproductive success (n = 2), limited food sources (n = 2), low natural abundance (n = 1), predation by crocodiles (n = 1), and introduced species (e.g. loricariid fishes in the San Juan river watershed) were also impacting sawfish populations in Costa Rica (Fig. 2B).

Table 3. Total largetooth sawfish records in major regions of Costa Rica where fishing gear and type of habitat was confirmed (n = 185). Only records that have associated data on fishing gear, habitat, or both are included in this table. 'Other' includes a variety of situations in which sawfish were reported: poisoned by an unknown chemical; poisoned or suffocated after a massive molasses spill; entangled in barbed wire, roots and tree branches; and found dead at the beach.

Fishing gear	Habitat type	Regions of Costa Rica					Total
		North Pacific	Central Pacific	South Pacific	Northern region	Caribbean	
Hook and line	Brackish		10	8			18
	Inshore		1	4		1	6
	Lagoon			2			2
	Offshore		2	1			3
	River	1	1	9	26	1	38
Gill/seine net	Brackish		35	5			40
	Inshore	7	8	2			17
	Lagoon				1		1
	Offshore	1	1	1			3
	River		1	1			2
Sighted	Brackish			2			2
	Inshore	1	1	1			3
	Lagoon				2		2
	Offshore	1		2			3
	River			1	6		7
Harpoon	Brackish		1				1
	River				14		14
Longline	Brackish		1	1			2
	Offshore		2	2			4
Trawl	Brackish		1				1
	Inshore		2	2			4
	Offshore		1	1			2
Other	Inshore		1				1
	River				9		9
Total		**11**	**69**	**45**	**58**	**2**	**185**

4. DISCUSSION

Based on our findings, the distribution and abundance of the largetooth sawfish *Pristis pristis* in Costa Rica has experienced a significant reduction, mainly due to fishing pressure in both coastal and riverine habitats. Nonetheless, we documented at least 2 hot spots in which recent sawfish sightings or captures were more common than in other areas: the TSNW in the South Pacific and the San Juan River watershed in the northern region. Sawfish had a broader distribution in the country than previously understood, including a wide range of freshwater, brackish, inshore and offshore habitats not previously reported as sawfish occurrence areas by Thorson (1976, 1982a,b), Winemiller (1983), Chicas-Batres (1995), Bussing (1998) and Hilje & Fournier (2017). These results highlight that some areas of Costa Rica may need special attention and that additional conservation efforts on key estuarine and freshwater habitats are needed in order to assist in recovery of populations of this critically endangered species.

Changes in the number of historical (>20 yr) and recent (<5 yr) records of largetooth sawfish in Costa Rica suggest there have been significant declines in both the Eastern Pacific and Western Atlantic subpopulations. The decline of largetooth sawfish in Costa Rica is consistent with global decline trends (Dulvy et al. 2014, 2016, Harrison & Dulvy 2014). Moreover, similar regional patterns have been described, as population reductions of largetooth sawfish have been reported in Mexico (Bonfil et al.2017), Nicaragua (Thorson 1982b), Colombia (Caldas et al. 2017), and Venezuela (Gómez-Rodríguez et al. 2014). The largetooth sawfish was declared locally extinct in Guatemala, Peru and Ecuador; however, recent records suggest there may be a population remnant in the latter 2 countries (Mendoza et al. 2017).

Our study showed that most historical records (>20 yr ago) were from the Gulf of Nicoya in the Central Pacific, one of the largest and most important estuaries of Costa Rica (Lizano & Vargas 1993, Vargas 1995). This estuary has been subject to intensive marine resource extraction (Lizano & Vargas 1993, Zanella et al. 2009) and is considered to have been overfished since the 1990s (Chacón et al. 2007, Tabash-Blanco 2007), which coincides with the time frame of most captures in this estuary. In this period ('>20 yr'), most sawfish captured in the Gulf of Nicoya were entangled in gill nets, which are still the most widely used fishing gear in the estuary (Chacón et al. 2007). According to fishers, some sawfish captured in this gulf during the rainy season were pregnant females, and, in some in stances, they aborted their fetuses when captured. This suggests that the Gulf of Nicoya and possibly the Tempisque River were likely a sawfish pupping ground.

Recent largetooth sawfish sightings are concentrated in 2 main regions. One of these is the northern region, specifically the San Juan watershed which represents a natural boundary between Nicaragua and Costa Rica. Together with Lake Nicaragua and the Colorado River, the San Juan watershed was considered to have one of the greatest freshwater concentration of sawfish in the world during the 1960s (Thorson 1982b). However, in the 1970s, an intense commercial netting fishery operating in Lake Nicaragua was responsible for a rapid decline of the largetooth sawfish population (Thorson 1982b). After the population was decimated, Nicaragua placed a 2 yr moratorium on the capture of sawfish in 1981 and an indefinite no-take status since 2002 (Thorson 1982b, McDavitt 2002, Ministerio del Ambiente y los Recursos Naturales 2002). Recent sawfish sightings along the border with Nicaragua may suggest a potential recovery of the population due to the no-take status in Nicaragua for >15 yr. Conversely, local enforcement of fishing regulations is still a major problem in this region as exemplified by the use of illegal fishing gears, such as harpoon and gillnets. Harpoon fishers in the San Juan River watershed target Atlantic tarpon *Megalops atlanticus* for commercial use, but due to limited visibility conditions, they likely kill sawfish incidentally; for example, nearly all recent sawfish records from this region were from harpoon fishers (Fig. 4). Furthermore, hook and line fishers reported 3 sawfish in 2018 from the San Juan River and 1 gillnet capture in April 2019 from a small Costa Rican tributary of the San Juan. These encounters highlight the need to conduct further research in this region.

Figure 4. (A) Largetooth sawfish killed by harpoon fishers in Boca Cureña in 2017, northern region of Costa Rica (unknown photographer). (B) Harpoon tip made out of a 3-sided file (picture by J. A. Valerio-Vargas). The file has been sharpened and grooves cut into it to hold to the flesh of tarpon or sawfish. This tip is fixed at the end of a long wood stick; once the harpoon is in the body of the animal, the tip detaches from the stick, and the animal can be traced by the floating plastic bottle acting as a buoy.

In addition to the San Juan River, recent largetooth sawfish records were documented in the TSNW region. This region holds one of the largest mangrove forests in Central America, comprising 17737 ha of protected mangrove area or 43% of all mangroves in Costa Rica (Mainardi 1996). While the TSNW does not hold a no-take status, it does offer protection to its mangrove forest and to certain sections of both major rivers, as regulations only allow small-scale subsistence agriculture and prohibit large-scale touristic or commercial developments (Ministerio del Ambiente y la Energía 2013). Collectively, these regulations have preserved sawfish habitats within the TSNW, by reducing threats such as pollution and synergistic effects from changes in land use due to deforestation for cattle grazing and extensive crops (e.g. pineapple and bananas). The vast extension of preserved sawfish habitats in the TSNW serves to explain the continuous sawfish records in this particular area. However, recently discovered illegal clearing of a marsh surrounding the Sierpe Lagoon, apparently for rice plantations in the upper reaches of the Sierpe River, uncontrolled fishing activities in surrounding in shore areas (J. A. Valerio-Vargas pers. obs.), and the loss of mangrove forest due to coastal sedimentation (Silva-Benavides et al. 2015) are threats that need to be addressed.

Sawfish meat has been reported to be sold in markets around the world (McDavitt 2002, 2014, Hossain et al. 2015, Leeney 2017). Based on our findings, sawfish meat was historically found (>20 yr) in some markets, sold primarily by gill net fishers. According to fishers from the Gulf of Nicoya, it was marketed under names such as ‘posta’, which could be translated to fish meat; ‘posta blanca’ (white fish meat); and ‘tiburón’ (shark). One retired fisher from the North Pacific of Costa Rica, mentioned that in the 1970s, a truck, mainly loaded with sawfish but also with shark meat, travelled weekly from Lake Nicaragua to fish markets in the capital of Costa Rica. Thorson (1982b) mentioned that sawfish meat was exported to several countries in Central America, although the countries were not mentioned.

Sawfish parts used in traditional medicine (e.g. respiratory ailments, vision problems, rheumatism, or general pain) were described from 9 countries by McDavitt (2014). Our findings on the use of sawfish parts in Costa Rican traditional medicine differ largely from those records. One of the main differences is that the most commonly mentioned medicinal use requires the physical action of the saw to obtain cure or relief from headaches and baldness, rather than the ingestion of a sub-product of the rostrum to obtain the supposed benefits.

The use of sawfish rostral teeth to fabricate cockfighting spurs has been mainly reported from South American countries (Kyne et al. 2013, McDavitt 2014, Ogden 2015); however, the present study provides the first evidence of this activity in Costa Rica. Although countries like Brazil, Ecuador, Panama, and several Caribbean nations are the main sources of sawfish rostral teeth, Costa Rica is possibly another major source of these valued items which find their way into the international cockfighting market. More recently, one of our team members was contacted by a dealer in Ecuador requesting sawfish rostral teeth for the cockfighting industry, which provided further evidence that illegal international trade may be common in the Latin American region. The illegal sawfish parts trade in Costa Rica, namely rostra and rostral teeth, represents a serious threat to the remnant sawfish populations, as the international market still creates a demand that may exert greater targeted fishing pressure to the already affected populations. Evidence of this illegal trade in Costa Rica demonstrates the need to monitor this type of activity, educate and create awareness among local authorities, and enforce international regulations such as CITES and CMS agreements. Local and regional efforts are urgently needed to address illegal sawfish trade.

Ongoing research efforts to define critical habitats for sawfish in Costa Rica, and the evidence that their populations have experienced significant declines in their distribution and abundance over the past 20 yr helped secure their legal protection by national authorities. The decree AJDIP/366-2017 (INCOPESCA 2017) protects both smalltooth *P. pectinata* and largetooth sawfish in Costa Rican waters in several ways: (1) a permanent fishing ban for both species, except for research purposes; (2) if a sawfish is caught incidentally, it must be reported to the authorities; and (3) sanctions will be applied if regulations are not followed. In November 2017, Costa Rica became one of 17 nations in which sawfish species have some level of legal protection. These measures respond to the urgently needed conservation efforts in its Eastern Pacific distribution (Kyne et al. 2013).

Knowledge about largetooth sawfish occurrence in riverine habitats and how local threats may affect the species' populations remains a top priority for a more comprehensive understanding of the global distribution of this species (Fernandez-Carvalho et al. 2014, Dulvy et al. 2016). According to Dulvy et al. (2016), 75% of the global historical largetooth sawfish distribution range is now regarded as presence uncertain (PU), which means the species was formerly known or thought to occur in the area, but it is no longer known if the species still occur. At a regional scale, part of the Central American countries were considered as PU (Dulvy et al. 2016); however, recent records from this study confirm the presence of the largetooth sawfish in Costa Rica, which is now part of the extant distribution of both the Eastern Pacific and Western Atlantic populations (Faria et al. 2013). Similarly, studies from 2017 and 2018 in data-poor regions (Mendoza et al. 2017, White et al. 2017, Leeney et al. 2018) have demonstrated the persistence of the species where it was thought to be extinct or PU. This shows that there are other beacons of hope outside of Australian protected areas where sawfish are found more frequently than any other country in their historical range. Nevertheless, even though there are some countries in the region where extinction has prevailed (Gómez-Rodríguez et al. 2014), we may still be in time to protect the species.

The finding of recent records and recognition of sawfish hotspots together with the achievement of legal protection measures for this Critically Endangered species gives hope for its conservation and serves to strengthen conservation actions in Costa Rica. Moreover, these outcomes will aid in international negotiations with Nicaragua and serve to increase enforcement cooperation now that both nations protect sawfish species. Back in the 1980s, Thorson (1982a) confirmed downstream and coastal movements of sawfish in the Lake Nicaragua-San Juan River system and Barra del Colorado surrounding areas in Costa Rica. This demonstrates that commercial netters at the river mouth could have an important effect on sawfish in the region; thus, sawfish conservation depends on actions from both nations. Finally, more than 30 yr after Thorson’s observation, both countries support the protection of sawfish, which could provide the means for a slow and long-term population recovery plan to save sawfish.

5. ACKNOWLEDGEMENTS

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CAPÍTULO 2

Identifying potential freshwater hotspots of the critically endangered largetooth sawfish in Central America using environmental DNA

Plate 2 Jorge Valerio filtering water samples at a field site in Humedal Nacional Térraba-Sierpe, south Pacific of Costa Rica, Capt. Esteban Jiménez in the back. Photo credit: Mario Espinoza.

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ABSTRACT

The use of environmental DNA (eDNA) has proven to be an effective approach in detecting rare and threatened species in a wide range of aquatic environments, and may become a valuable conservation tool in ecosystems with high suspended sediment content. Therefore, eDNA can be useful to identify critical habitats for threatened organisms such as the largetooth sawfish (*Pristis pristis*), an euryhaline-generalist species, once common in Central America. In Costa Rica, knowledge of *P. pristis* distribution relies mainly on local ecological knowledge, which may not represent the species actual distribution due to their low abundance and cryptic habits in turbid environments. This study assessed the application of eDNA for the detection of *P. pristis* in Costa Rica through a country-wide survey. Environmental DNA samples were collected *in situ* by filtering water through filter membranes using a portable filtration device. Laboratory analysis was carried following the protocols used by the Global Sawfish Search project, developed at the James Cook University, Australia. A total of 579 samples were collected at 93 sampling stations in 18 sites within six main regions of the country. *P. pristis* eDNA was detected from 16 samples collected from the Northern region and Northern Caribbean region, along the San Juan-Colorado River. This study provided the first direct evidence of *P. pristis* in Central America using eDNA, further demonstrating that eDNA can detect rare species in fast flowing waters with high suspended sediment content. Moreover, detections of *P. pristis* combined with recent catch/release records from the San Juan-Colorado River suggest their numbers are slowly increasing. Urgent cooperative research is needed to evaluate their population in this freshwater system. An ecosystem approach to manage the system is suggested to secure their populations, by decreasing threats and allow for a stable recovery of a historical core population of their Western Atlantic subpopulation.

KEY WORDS

Pristis pristis; Costa Rica; San Juan-Colorado River; critical habitats; eDNA surveys; mitochondrial DNA; Western Atlantic subpopulation

1. INTRODUCTION

The study of the distribution of aquatic endangered species presents serious challenges when dealing with cryptic, bottom-dwelling species that occur in very low numbers (Bellemain et al. 2016; Simpfendorfer et al. 2016). Moreover, traditional survey methods such as underwater visual census and fishing techniques may also fail to detect rare species that live in estuarine and freshwater environments with elevated suspended sediment loads (Simpfendorfer et al. 2016; Wilcox et al. 2016), thus creating an urgent need for developing novel and specialized approaches capable of overcoming such circumstances. The use of environmental DNA (eDNA), a method that relies on the detection of DNA traces in the environment, has proven to be a powerful ecological tool in the detection of a variety of rare and threatened aquatic organisms in a variety of environments (Dejean et al. 2011; Laramie et al. 2015). Given the rapid degradation of eDNA in aquatic environments, a positive detection depicts the actual presence of the species in a particular environment (Barnes et al. 2014). Therefore, the use of eDNA is becoming a valuable conservation tool to identify critical habitats for threatened species in poorly studied aquatic environments such as large tropical rivers and estuarine habitats with high suspended sediment content (Syvitski et al. 2014; Bellemain et al. 2016; Huerlimann et al. 2020).

The largetooth sawfish (*Pristis pristis*) is a bottom-dwelling ray (elasmobranch) with a former wide tropical distribution in freshwater, inshore and estuarine environments (Faria et al. 2013; Kyne et al. 2013). *P. pristis* may attain sizes of up to 6.5 m total length (Last et al. 2016), and is characterized by a shark-like body and a highly modified rostrum with 14 to 23 rostral teeth per side (Faria et al. 2013; Last et al. 2016). The species has suffered globally from fishing and habitat degradation, leading to significant population declines and a reduction in their extent of occurrence, and is now listed as critically endangered based on global assessments by the International Union for the Conservation of Nature (IUCN) (Kyne et al. 2013; Dulvy et al. 2016; Yan et al. 2021). *P. pristis* has four subpopulations worldwide, including the Eastern Atlantic, Western Atlantic, Eastern Pacific and Indo-West Pacific (Faria et al. 2013). Sawfish from the Western Atlantic and Eastern Pacific subpopulations were historically found in the Central American region (Thorson et al. 1966; Thorson 1982b; Cook et al. 2005; Kyne et al. 2013); however, their presence is currently uncertain in some areas with historical records of the species due to the lack of recent studies and severe depletion due to overfishing (Dulvy et al. 2016; Yan et al. 2021).

Although the status of *P. pristis* remains largely unknown in Central America, a recent study in Costa Rica suggested that there are two potential hotspots for the species, one in the south Pacific and another in the northern region (Valerio-Vargas & Espinoza 2019). Specifically, Valerio-Vargas & Espinoza (2019) identified the vast mangrove and estuarine/riverine habitats of the Térraba-Sierpe National Wetlands (TSNW) in the south Pacific region and the freshwater ecosystems of the San Juan River basin in the northern region of Costa Rica as areas where the abundance/sightings of the species appeared to be more common. This information was mainly gathered through local ecological knowledge and few landing records, which may limit our understanding of the actual distribution of *P. pristis* due to their low abundance and concealment within highly turbid environments (Wiley & Simpfendorfer 2010; Simpfendorfer et al. 2016). Moreover, there is a wide range of potential sawfish habitats in remote areas of Costa Rica with little disturbance, including mangroves, wetlands, estuaries, large rivers and coastal lagoons; highlighting the need to identify key areas of the country where management and conservation actions could be implemented to ensure persistence of both of these subpopulations.

In this study, we assessed the application of eDNA at detecting the presence of the critically endangered *P. pristis* in Costa Rica. By using a stratified sampling approach through the entire country, we were able to survey (i) areas with historic and recent sawfish records (Thorson 1982a; Bussing 1998; Valerio-Vargas & Espinoza 2019), and (ii) areas with suitable habitats for sawfish that lacked any sighting or landing records. The use of eDNA allow us to identify specific habitats where we can prioritize further conservation efforts in Costa Rica, which is one of the range states identified as a priority region by the Species Specialist Commission and the Shark Specialist Group of the IUCN (Harrison & Dulvy 2014; Fordham et al. 2018). We also tested for the presence of the smalltooth sawfish *P. pectinata* in samples collected from coastal environments of the Caribbean Sea, given the historical occurrence of the species throughout the Caribbean and their potential to re-expand into the region following signs of recovery of the population in the south-east United States (Brame et al. 2019).

2. METHODS

Study region

Costa Rica is a small country (approx. 51100 km^2) of Central America, which is located between Nicaragua in the north and Panamá in the south (Cortés & Wehrtmann 2009). The Pacific of Costa Rica has a long (1254 km) and highly irregular coastline characterized by numerous bays, gulfs and large estuaries (e.g. Golfo de Nicoya, Golfo Dulce and the TSNW). Most of the mangrove cover (99%) is found in the central and south Pacific regions, mainly due to the discharge of large rivers and an intense rainy season (Jiménez & Soto 1985; Jiménez 1999). The Caribbean coastline is relatively short (212 km) and less irregular, with fewer mangrove patches located mainly in the south (Gandoca-Manzanillo); there are some coastal lagoons associated with large rivers such as the San Juan-Colorado, Pacuare, Matina and Sixaola (FAO 2004; Cortés & Wehrtmann 2009). The northern region of Costa Rica is characterized by a vast network of aquatic environments including the Sarapiquí, San Carlos and Frío rivers (Barrientos & Chaves 2008; Rojas 2011), which form a continuum with Lake Nicaragua and its outflow, the binational San Juan-Colorado River. This large river diverges and empties in the Caribbean Sea at two locations: (i) San Juan del Norte in Nicaragua, and (ii) Barra del Colorado in the Northern Caribbean region of Costa Rica (Thorson 1982b).

Site selection

Four criteria were used to select sampling sites for eDNA surveys: (i) sites that were located in or near the two potential sawfish hotspots identified by (Valerio-Vargas & Espinoza 2019); (ii) sites that were historically important for sawfish based on previous studies and local ecological knowledge (Thorson 1982b; Angulo et al. 2013; Valerio-Vargas & Espinoza 2019); (iii) sites with no available sawfish records, but that offered suitable habitats for the species, such as mud-sandflats, mangrove-lined estuaries, shallow (<10 m) bays and rivers, some of these within protected areas; and (iv) other sites from which we had recent sawfish reports of captures and/or landings. Based on these criteria, a total of 18 sites within six main regions of Costa Rica were chosen to conduct our eDNA surveys: northern Pacific, central Pacific, southern Pacific, northern region, northern Caribbean and southern Caribbean (Fig. 1; Table 1).

P. pectinata was known to occur historically throughout the Caribbean (Carlson et al. 2013), where Thorson (1982b) and Valerio-Vargas & Espinoza (2019) reported the species from Costa Rican waters. Therefore, we also tested for *P. pectinata* eDNA, from the samples collected at the coastal lagoons and lower reaches of the Colorado River in Barra del Colorado (northern Caribbean), and the lower reaches of the Sixaola River (southern Caribbean) (Fig. 1). The Gandoca Lagoon in the southern Caribbean (Fig. 1) was not tested for *P. pectinata* since it is only temporally connected with the Caribbean, and it was recently and barely opened by the time of the sampling. Site-specific information regarding protection status, type of environment and season is summarized in Table 1.

Using satellite images of selected sites, we initially established an array of sampling stations following a systematic stratified design concept, in which stations were fixed, equidistantly separated within sites. The number of stations and distance from each other was set depending on the size and relative importance of each sampling site, as we aimed to maximize sampling area coverage across regions. Therefore, distance between sites varied considerably from 150 m to 8.5 km in some environments. Once in the field, the specific location of the sampling stations was refined, based on traditional ecological knowledge, potential suitable habitats, and recent capture/sighting locations. All sites were sampled from February to August 2019, during both dry (November to April) and wet (May to October) seasons. Some stations in the San Juan River were sampled in March (dry season) and others in May (wet season).

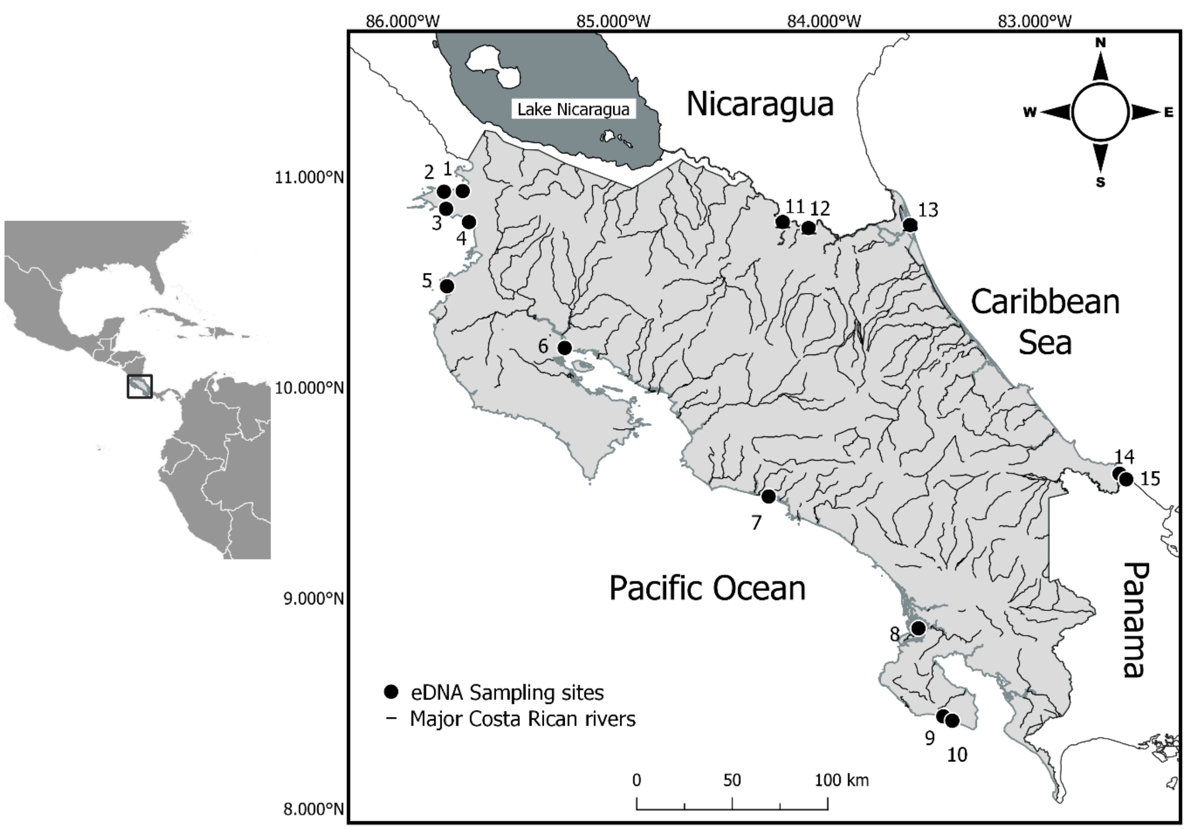

Figure 1. Environmental DNA (eDNA) sampling sites selected throughout Costa Rica, Central America. (1) Bahía Thomas; (2) Bahía Santa Elena; (3) Bahía Potrero Grande; (4) Bahía Naranjo; (5) Playa Danta y Dantita; (6) Boca Tempisque; (7) Estero Palo Seco; (8) Humedal Nacional Térraba-Sierpe; (9)

Laguna Pejeperrito; (10) Laguna Pejeperro; (11) Boca San Carlos; (12) Boca Cureña-Cureñita; (13) Río Colorado/Barra del Colorado sites (includes 3 sites in rivers, coastal lagoons and river outlet); (14) Laguna Gandoca; (15) Río Sixaola. Sites 13 and 15 were tested for *P. pectinata* eDNA

Table 1. Summary of environmental DNA (eDNA) samples collected in different habitats across regions and sites of Costa Rica, Central America. Sampling site indicates the geographic areas that were sampled; No. stations indicate the number of stations that were sampled within each sampling site.

Region	Sampling Site	Protection status	Environments	Season	No. stations	No. samples	Samples by region
Northern Pacific	Bahía Santa Elena	Marine Management Area[a] (since 2018)	Mangroves, intertidal mud/sand flats	Dry	7	35	161
	Bahía Thomas	None	Intertidal Mud/sand flats and sand bottom	Dry	2	10	
	Bahía Potrero Grande	National Park[b] (since 1971)	Mangroves, intertidal mud/sand flats	Wet	12	67	
	Bahía Naranjo		Sand bottom (<10 m)	Wet	3	19	
	Playas Danta and Dantita	None	Inshore, sand bottom (<10 m)	Dry	5	30	
Central Pacific	Boca Tempisque	None	Estuary, mud bottom (<10 m)	Dry	4	20	55
	Estero Palo Seco	None	Mangroves, intertidal mud/sand flats (<10 m)	Dry	7	35	
Southern Pacific	Humedal Nacional Térraba-Sierpe	Forest Reserve, National Wetland[c] (since 1994)	Mangroves, intertidal mud/sand flats (<10 m)	Dry	11	55	123
	Laguna Pejeperrito	Lacustrine Wetland[d] (since 1994)	Coastal lagoon, brackish, no mangrove	Wet	6	30	

	Laguna Pejeperro	Wildlife Refuge[e] (since 1998)	Coastal lagoon, brackish, mangrove-lined	Wet	7	38	
Northern region	Río San Juan, Boca San Carlos	Biological Reserve[f] (Nicaraguan)	River	Dry and wet	4	20	55
	Río San Juan, Boca Cureñita		River	Dry and wet	7	35	
Northern Caribbean	**Río Colorado, Barra del Colorado***	Wildlife Refuge[g] (since 1985)	River and inshore	Dry	4	20	60
	Laguna Agua Dulce, Barra del Colorado*		Coastal lagoon, brackish, no mangrove	Dry	1	5	
	Laguna De Atrás, Barra del Colorado*		Coastal lagoon, brackish, no mangrove	Dry	3	15	
	Río Colorado, Dos Bocas		River	Dry	4	20	
Southern Caribbean	Laguna de Gandoca	Wildlife Refuge[h] (since 1985)	Coastal lagoon, brackish, mangrove-lined	Wet	3	17	32
	Río Sixaola*	None	River	Wet	3	15	
Total samples					**93**	**486**	**486**

*Sampling sites that were also screened for *P. pectinata* eDNA

[a] Bahía Santa Elena Marine Management Area: marine zoning is applied in this site, with no fishing permitted near the mangroves and only small-scale hook and line fishing in other zones of the bay (SINAC 2017a)

[b] Santa Rosa National Park: No-take zone (SINAC 2013)

[c] Térraba Sierpe National Wetlands: protection is offered to its mangrove forest and to certain sections of both Térraba and Sierpe rivers, as regulations only allow small-scale subsistence agriculture, prohibit large-scale touristic or commercial developments, and only allow small-scale hook and line fishing (MINAE 2013)

[d] Pejeperrito Lacustrine Wetland: no explicit fishing regulations are stated in the protection document (MIRENEM 1994), but due to the lack of a management plan for this body of water, fishing is illegal in this lagoon.

[e] Pejeperro National Wildlife Refuge: no explicit fishing regulations are stated in the protection document (MINAE 1998), but due to the lack of a management plan for this body of water, fishing is illegal in this lagoon

[f] Reserva de Biosfera del Sureste de Nicaragua: this reserve encompasses almost the entire San Juan River on the Nicaraguan side; no fishing is allowed (MARENA 1999)

[g] Barra del Colorado Wildlife Refuge: regulations only allow small-scale subsistence agriculture, prohibit large-scale touristic or commercial developments, and only allow small-scale hook and line fishing (SINAC 2012); fishing regulations in the inshore environment are currently being instantiated through a marine zoning plan (JAVV pers. obs. 2021).

[h] Gandoca-Manzanillo Wildlife Refuge: marine zoning is applied in this site, only small-scale hook and line fishing is permitted in the lagoon (SINAC 2017b)

Sampling and equipment

Field sampling was executed following protocols developed in Cooper et al. (2021). Samples from the field stations surveyed were collected from an anchored boat. Before the sampling procedure, we recorded latitude/longitude (Garmin GPSMAP 62s), depth (Hondex depth meter), turbidity (Secchi disc), and flow speed (slow, moderate or fast). A handheld multimeter (EcoSense EC300A) was used to record water temperature and salinity. Nearly 10% of temperature measurements were collected with a data logger (HOBO UA-002-08) due to multimeter failure; no data on salinity was recorded in those same instances.

At each sampling station a total of 5 replicate 5 l water samples were collected, plus a negative control. A total of 579 samples were collected across 93 sampling stations, of which 486 were used for detecting *P. pristis* (55 of these samples were also used for detecting *P. pectinata*) and 93 were control. The membrane pore size varied between 5 and 20 µm, and its pore size was selected to allow the proposed amount of filtrate in each scenario, in dependence of water turbidity; therefore, murkier water was filtered with greater pore size than clearer water (Turner et al., 2014; Simpfendorfer et al., 2016; Cooper et al., 2021). Where time and turbidity constraints allowed, extra samples were collected using a 1.2 µm filter membrane. Information on the filtrate amount, membrane pore size, and total filtering time was recorded for each replicate. Following filtration, each filter was rolled and folded in half using sterile forceps and scissors and placed inside a 2 mL LoBind microtube containing 1.5 mL of Longmire's buffer solution. Samples were stored at ambient temperature during the survey period and shipped to James Cook

University, Queensland, Australia, where they were stored at ambient temperature until extraction in a dedicated laboratory.

Prior to sample collection, the working area in the boat and sampling equipment were sterilized by applying 10% bleach solution with gloves and a saturated paper towel, leaving to a stand for 5 minutes, followed by cleaning with distilled water using a moistened paper towel.

Laboratory analysis

Extraction and purification of total eDNA was completed following a glycogen-aided precipitation extraction method, described in Edmunds and Burrows (2020) with additional modifications for filter papers stored in 2 mL microtubes (Cooper et al. 2021). Presence-only detection of *P. pristis* and *P. pectinata* eDNA was conducted using species-specific TaqMan quantitative PCR (qPCR) assays on Applied Biosystems QuantStudio 5 Real-Time PCR System (Life Technologies, ThermoFisher Scientific Pty Ltd, Victoria, Australia) (Cooper et al., 2021). A TaqMan Exogenous Internal Positive Control (IPC) qPCR assay (Applied Biosystems; Hartman, Coyne, & Norwood, 2005) with a custom internal probe modification (i.e., ABY-QSY) was used to test for qPCR inhibition, as per the manufacturers protocol. Putative positive amplicons were sent to the Australian Genome Research Facility (AGRF) for bidirectional Sanger sequencing.

Data analysis

Data on fluorescence were analyzed using QuantStudio Analysis Software version 1.4.2. Sequence data were aligned in Geneious version 10.2.6 and species identity confirmed using the BLAST function against the GenBank NCBI database. Inter-species sequence variation was analyzed by visual assessment of aligned sequence data in Geneious. Detections were only considered as true positive detections if (i) the retrieved sequence a >97% match with the sawfish species reference sequences, and (ii) when the corresponding negative controls showed no amplification (Cooper et al., 2021).

To examine the spatial distribution of sawfish across sampling sites and stations, we plotted all confirmed positive records using QGIS 3.18 (QGIS.org, 2021). These maps provided additional information on the potential connectivity of sawfish between habitats, which remains crucial for the conservation of this species in Costa Rica.

3. RESULTS

eDNA detection

DNA of *P. pristis* was successfully extracted, amplified and sequenced from samples collected at two of the six sampled regions (Fig. 2) (Table 2). Overall, *P. pristis* was detected in 16 of the 486 samples collected, which represents 3.3% success rate across the entire country. Positive samples were collected with 20 µm filter membranes in 10 of the 93 sampling stations. There were no detections of *P. pectinata* in any of the sampling sites.

P. pristis was detected in sampling stations from the Northern region and Northern Caribbean region (Fig. 2), which suggest some level of connectivity within the San Juan-Colorado River basin. Detections in the northern region (Fig. 2A) occurred along the main channel of the San Juan-Colorado River, whereas in the Northern Caribbean region (Fig. 2B), they occurred both just before the river mouth and in the inshore environment (Fig. 2). In the northern region, Boca San Carlos and Boca Cureña-Cureñita (Fig. 2A) had 9 and 5 confirmed positive detections, respectively, whereas in the Northern Caribbean region there were only 2 detections in Barra del Colorado (Fig. 2B). In Boca San Carlos, detections were distributed in 3 of the 4 sampling stations, in Boca Cureña-Cureñita, in 5 of the 7 stations, and in Barra del Colorado in 2 of the 12 stations (Fig. 2). Sampling stations in these environments, were characterized by freshwater conditions, apparent fast flowing waters, high turbidity, and water temperatures ranging from 27.6-29.9° C. All of these sites were in relatively shallow areas (<4 m), with the exception of a deep pool (9 m) in Boca Cureña-Cureñita (San Juan River, Northern region) (Table 3).

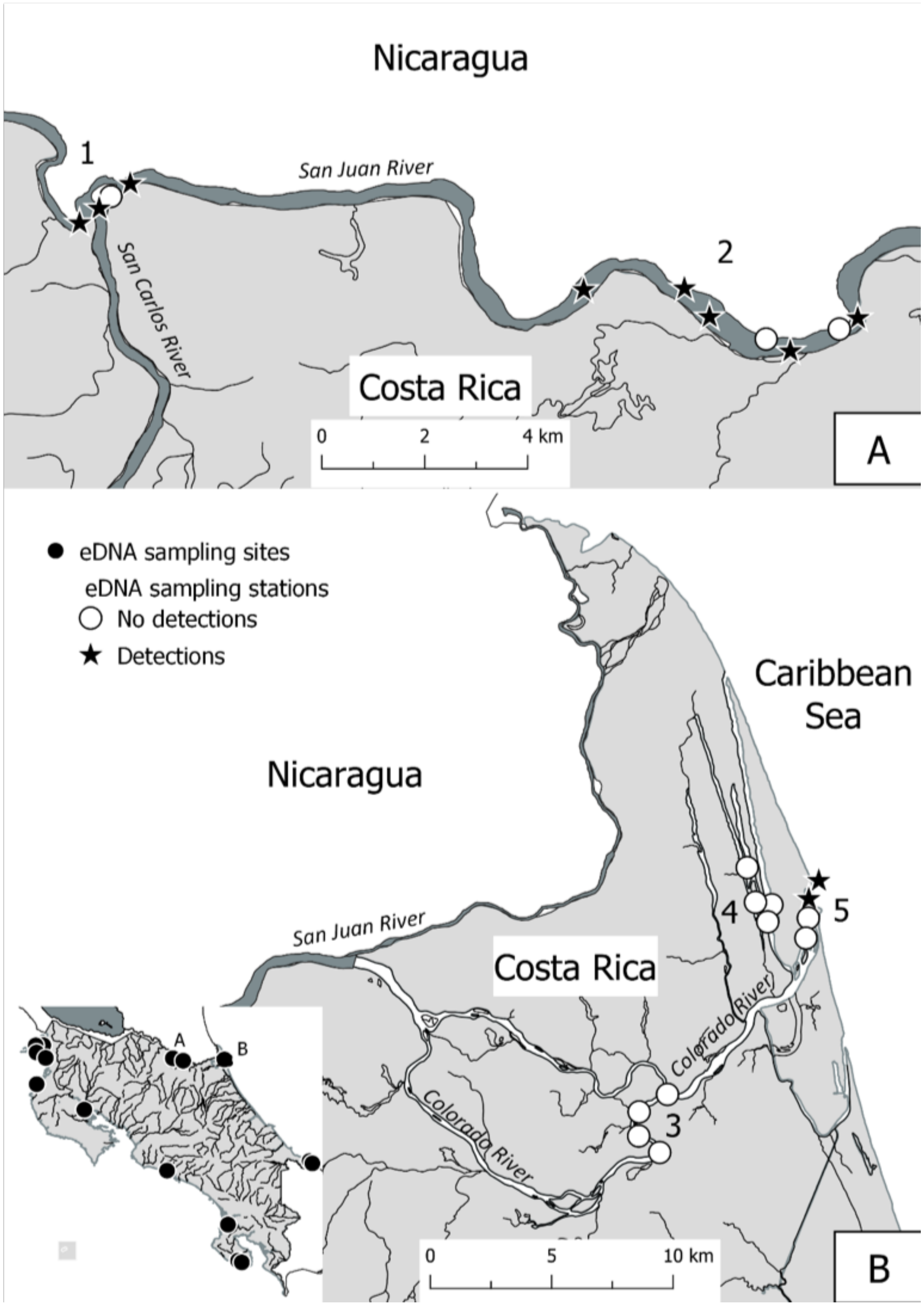

Figure 2. eDNA sampling sites and stations in which sawfish was detected. **A** Northern region: 1) Boca San Carlos sampling stations; 2) Boca Cureña-Cureñita sampling stations. **B** Northern Caribbean region: 3) Río Colorado, Dos Bocas sampling stations; 4) Laguna Agua Dulce and Laguna De Atrás sampling stations; 5) Río Colorado, Barra del Colorado sampling stations.

Table 2. Positive *P. pristis* eDNA samples from regions and sites of Costa Rica.

Region	*Sampling site*	*No. stations*	*No. samples*	*Positive samples by site*
Northern Pacific	Bahía Santa Elena	7	35	0
	Bahía Thomas	2	10	0
	Bahía Potrero Grande	12	67	0
	Bahía Naranjo	3	19	0
	Playas Danta and Dantita	5	30	0
Central Pacific	Boca Tempisque	4	20	0
	Estero Palo Seco	7	35	0
Southern Pacific	Humedal Nacional Térraba-Sierpe	11	55	0
	Laguna Pejeperrito	6	30	0
	Laguna Pejeperro	7	38	0
Northern region	Río San Juan, Boca San Carlos	4	20	9
	Río San Juan, Boca Cureña-Cureñita	7	35	5
Northern Caribbean	Río Colorado, Barra del Colorado	4	20	2
	Laguna Agua Dulce, Barra del Colorado	1	5	0
	Laguna De Atrás, Barra del Colorado	3	15	0
	Río Colorado, Dos Bocas	4	20	0
Southern Caribbean	Laguna de Gandoca	3	17	0
	Río Sixaola	3	15	0
Total		**93**	**486**	**16**

Table 3. Environmental data from positive eDNA samples of Costa Rica.

Region	*Sampling site*	*Sampling station*	*Flow*	*Turbidity (m)*	*Water temperature (°C)*	*Water depth (m)*	*Salinity (ppt)*
Northern region	Boca San Carlos	S1-BSC-R1	slow	0.45	29.9	2	0
		S2-BSC-R2	fast	0.25	27.6	0.3	0
		S4-BSC-R4	slow	0.7	28	2	0
	Boca Cureña-Cureñita	S1-BCU-R1	fast	0.35	28	1.5	0
		S2-BCU-R2	fast	0.4	28	1.2	0
		S4-BCU-R4	fast	0.35	28.4	3.4	0
		S6-BCU-R6	fast	0.5	28	9	0
		S7-BCU-R7	fast	0.3	27.8	1.4	0
Northern Caribbean	Barra del Colorado	S1-BCO-E1	moderate	0.5	28.2	1.5	0.1
		S2-BCO-E2	fast	0.5	28.4	3.4	0.1

DNA sequences

P. pristis sequences (Appendix S1) from the San Juan-Colorado River basin showed a single base-pair transition (T to C) of the mitochondrial *12S* gene (Fig. 3). This single base-pair transition appears to be characteristic of the samples from this study, as it is absent from *P. pristis* sequences obtained from Australian and Papua New Guinea tissue samples (unpublished data).

4. DISCUSSION

Understanding the true extent of the distribution of the largetooth sawfish *P. pristis* is crucial to identify priority areas for conservation, particularly in freshwater ecosystems where a paucity of data prevails. This study provides: (1) the first direct records of the critically endangered *P. pristis* in freshwater environments of Central America based on a stratified eDNA sampling survey; (2) the first genetic sequences of *P. pristis* obtained from the Lake Nicaragua-San Juan River system, and one of the few genetic samples from the Western Atlantic (WA) subpopulation of *P. pristis*; and (3) sufficient evidence that the analysis of eDNA is sensitive enough to detect rare species in fast flowing waters with high suspended sediment content, thus providing a useful ecological tool for the conservation of threatened aquatic species. The latter is of particular importance, as the protocol could aid in providing detailed information on the distribution of sawfishes in other freshwater and brackish environments around the world, specially by using species specific primers that will aid in avoiding species uncertainty (Cooper et al. 2021), a problem recently encountered by Bonfil et al. (2021) in Mexico. Collectively, given our findings of *P. pristis*' persistence in the San Juan-Colorado River since seminal research on this population four decades ago (Thorson 1976, 1982b, 1982a), it remains crucial to conduct further research to estimate the population size, life history parameters, movements, and identify critical habitats of *P. pristis* at remote areas of Costa Rica. Furthermore, promoting education and raising awareness around this critically endangered species has proven to be key components in multiple conservation and recovery efforts globally (Fordham et al. 2018).

The use of eDNA successfully detected *P. pristis* in several stations from the northern and the north Caribbean regions of Costa Rica, which are located within the basin of the binational San Juan-Colorado River. Our findings support the interpretation of Valerio-Vargas & Espinoza (2019), where Boca San Carlos and Boca Cureña-Cureñita in the northern region of Costa Rica were identified as a potential "sawfish hotspot". This is further supported by the fact that part of the sampling in Boca San Carlos and Boca Cureña-Cureñita were conducted during the dry and rainy season, with samples from both seasons providing *P. pristis* detections. Results from Barra del Colorado (north Caribbean region) showed the species is also present in this river section, suggesting that the entire San Juan-Colorado River could be acting as a corridor for the species, and thus, coordinated conservation efforts between countries (Costa Rica–Nicaragua) are crucial to the recovery of species. Moreover, between 2020–2021 there has been multiple captures of juvenile *P. pristis* along the San Juan-Colorado River, which is another line of evidence that this entire region may play a key role in future sawfish conservation efforts (Fig. 3–4). Therefore, the San Juan-Colorado River appears to be of cornerstone significance, not only as one of the last remaining areas where

P. pristis may still be found in the Central American distribution of its Western Atlantic subpopulation, but also as an area with a reproductive and viable population (Fernandez-Carvalho et al. 2014).

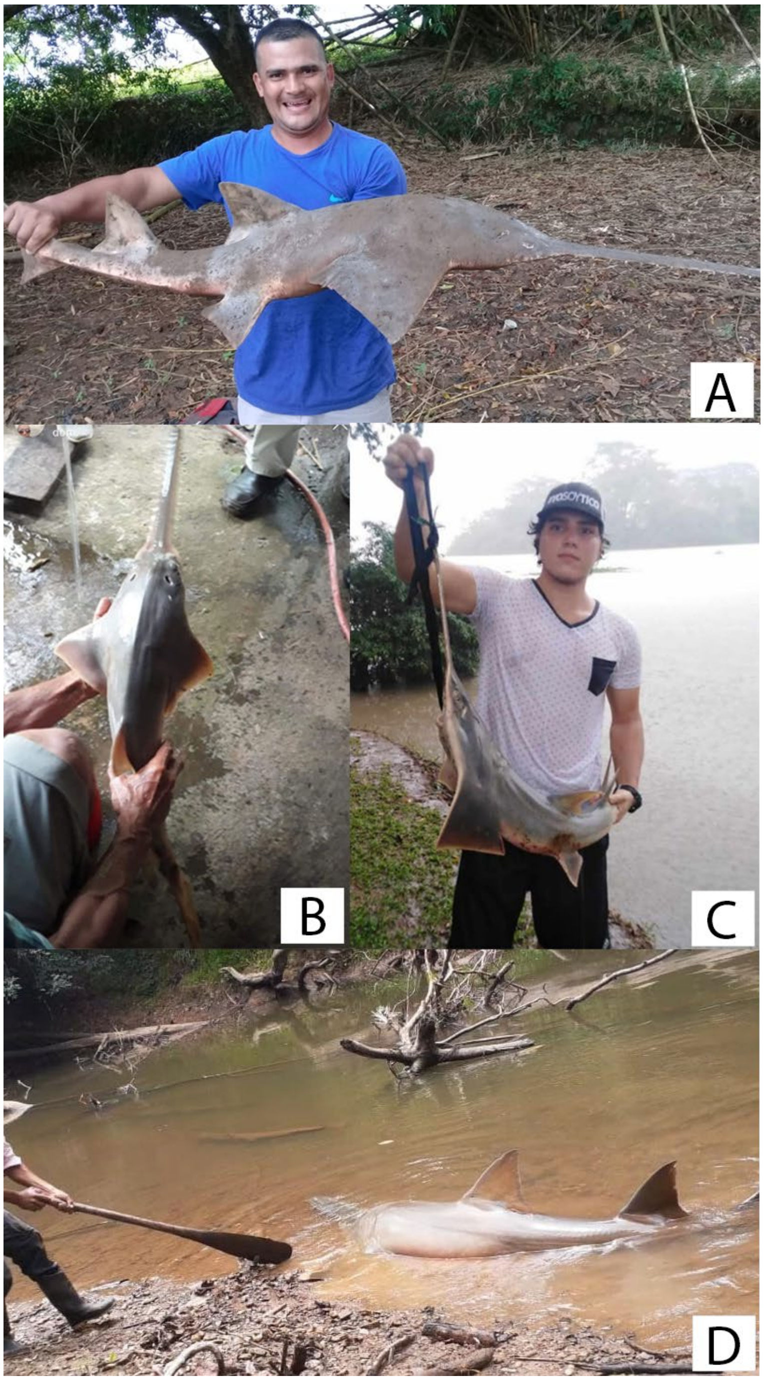

Figure 3. Recent captures of *P. pristis* along the San Juan River. A: juvenile (<1.5 m) caught at Remolinito in March 2020 (photo credit Marlon Oporta); B: newly born (<1 m) caught at Boca San Carlos in September 2020 (photo credit Miguel Su); C: juvenile (<1.5 m) caught at Boca San Carlos in November 2020 (photo credit Oscar Carranza); D: adult (2.5 – 3 m) caught near the Machuca rapids in June 2021 (photo credit Bryant Reyes). All sawfish were released alive and unharmed.

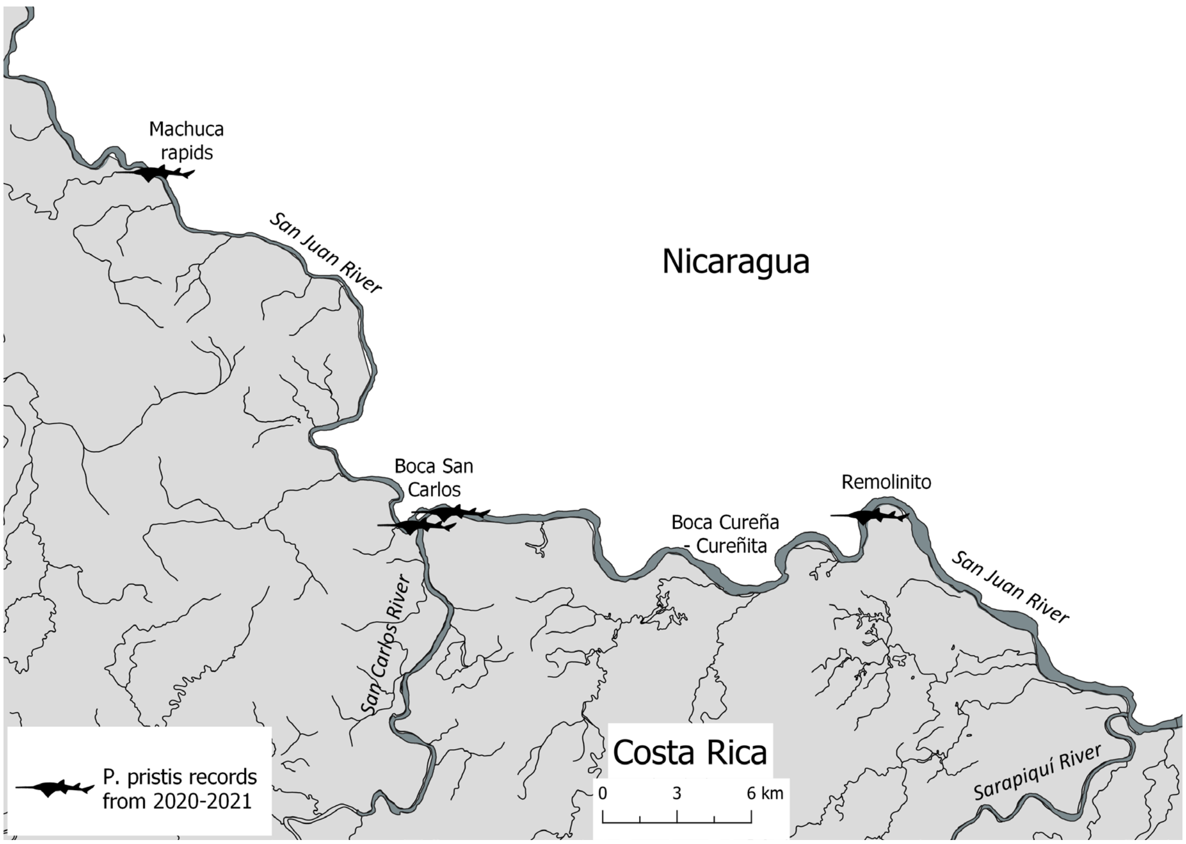

Figure 4. Location of recent captures of *P. pristis* along the San Juan River. Remolinito: juvenile (<1.5 m) caught in March 2020; Boca San Carlos: newly born (<1 m) caught in September 2020 and juvenile (<1.5 m) caught in November 2020; Machuca rapids: adult (2.5 – 3 m) caught in June 2021. Positive eDNA samples were collected from Boca San Carlos and Boca Cureña-Cureñita.

In the Southern Caribbean region, we did not find detections of *P. pristis* in samples from the Gandoca lagoon or the Sixaola River, although Valerio-Vargas & Espinoza (2019) showed recent records from one *P. pristis* and one *Pristis sp*. in this region. To our knowledge, sawfish are absent from every ichthyological study conducted in the Gandoca lagoon (e.g. McLarney 1988; Chacón-Chaverri 1994; Zúñiga 2005; Benavides Morera & Brenes 2010). Moreover, in over 30 years of research in this area, no sawfish were ever recorded or heard of from inside the lagoon (W. McLarney pers. comm., 2019; D. Chacón, pers. comm., 2021), and only one record (>20 years) from the Sixaola River, near the community of Bonife, <10 km from the river mouth (W. McLarney pers. comm., 2019), though it seems sawfish were not particularly abundant in this region (Valerio-Vargas & Espinoza 2019). The Gandoca lagoon has the largest and most developed mangrove forest in the Costa Rican Caribbean, mainly composed of red mangrove (*Rhizophora mangle*) (Coll et al. 2001), and declared of international significance by Ramsar since 1996 (Windevoxhel et al. 1995). Even if the lagoon presents apparent suitable sawfish habitat, its connection with the Caribbean Sea is highly unstable, as the sandbar that encloses the lagoon, opens and closes on a monthly or even weekly basis due to oceanographic and climatological factors (Coll 2000). Accessibility to mangroves is key in determining their potential role as a refuge or feeding habitat for fish species, as it enables biological connectivity between adjacent ecosystems (Sheaves 2005). Several estuarine fish species (e.g. snook, drum, mojarra) (Benavides Morera & Brenes 2010) occur in the lagoon, and it is also an important nursery for the Atlantic tarpon (*Megalops atlanticus*) (Chacón-Chaverri 1994); hence, there is sufficient biological connectivity with the sea to allow access for certain diadromous species. It seems that a combination of low abundance in the area, together with the collapse of their populations in the 1980's and the unstable accessibility to the lagoon could explain the lack of historical sawfish records and eDNA detections. The nearby San San Pond Sak wetland, specifically the San San lagoon, is a mangrove estuary that could present another potential sawfish habitat. It presents vast red mangrove areas and uninterrupted connection with the Caribbean (Gonzalez-Socoloske et al. 2015), however, we are unaware of any sawfish records from this environment. Sawfish research efforts in Panama have focused on the Pacific coast (López-Angarita et al. 2021), leaving an important area for future studies.

In the Costa Rican Pacific, recent sawfish records are found scattered along the coast (Valerio-Vargas & Espinoza 2019), mainly in the Térraba-Sierpe National Wetland (TSNW), but also in or near other of our eDNA sampling sites (e.g. Bahía Thomas, Boca Tempisque, Estero Palo Seco) (Table 1, Fig. 1). However, we are aware of two other confirmed records from 2020 in the Northern Pacific region, one of them near the Playa Danta-Dantita sampling site (Table 1, Fig. 1). Several ecological aspects could have limited our ability to detect *P. pristis* from eDNA surveys conducted in the Pacific of Costa Rica. There are major ecological differences between the San Juan-Colorado River basin and Pacific coast environments (Abell et al. 2008; Cortés & Wehrtmann 2009; Herrera 2016), which may cause important differences in the behaviour and habitat use of *P. pristis*. In the San Juan-Colorado basin, heavy rains provide a continuous freshwater supply flowing from Lake Nicaragua (Abell et al. 2008); there are also no mangroves, and the tide amplitude throughout the Costa Rican Caribbean is low (<50 cm) (Cortés & Wehrtmann 2009). Apparently, these and other ecological factors have influenced sawfish behaviour in the San Juan-Colorado basin, causing them to spend all or most of their lives confined to freshwater habitats, with only some individuals moving downstream to coastal environments (Thorson 1982b). In contrast, the Pacific coast is characterized by marked seasonal and spatial differences in precipitation and freshwater flow (Abell et al. 2008; Cortés & Wehrtmann 2009; Herrera 2016), which may be important drivers of seasonal movement and migratory patterns.

To our knowledge, there is no available information regarding seasonality in their EP subpopulation, with the only exception being Valerio-Vargas & Espinoza's (2019) consideration that the Gulf of Nicoya and Tempisque River were possibly a sawfish pupping ground during the rainy season. This phenomenon could also occur in other river mouths and estuaries with suitable sawfish habitat, such as most of our other Pacific coast sampling sites (Table 1, Fig. 1). With the exception of the Tempisque river mouth, samples from the northern Pacific (i.e. Bahía Thomas, Bahía Santa Elena, Bahía Potrero Grande, Bahía Naranjo, Playa Danta-Dantita) were collected during what normally constitutes the wet season in that region (Herrera 2016); however, a strong precipitation deficit affected the Costa Rican Pacific coast during the sampling period (IMN 2019a). Samples from the Central Pacific in the Palo Seco estuary and from the Southern Pacific in the TSNW were collected during the dry season, whereas the Pejeperro-Pejeperrito coastal lagoons were sampled during a deficient wet season (IMN 2019b).

According to Peverell (2009), sawfish from the WP subpopulation appear to use the freshwater flow and rain patterns as environmental cues that trigger their pupping, which in Australia's NT, seems to occur late in the wet season. Therefore, it is likely that the climatic variation could have had an important effect on sawfish using coastal and estuarine habitats.

Based on the ecological data of *P. pristis* from the Indo-West Pacific (Whitty et al. 2008, 2017; Lear et al. 2019) and recent findings from Costa Rica (Valerio-Vargas & Espinoza 2019), it is likely that eDNA samples collected across environments are targeting different life-stages of the species. Samples taken from rivers and estuaries would likely be targeting juveniles and sub-adults, and when taken in the rainy season, could also target pupping females, whereas samples from inshore waters could be targeting both adults and sub-adults, and in offshore environments, only the former. Another factor to consider is their reproductive periodicity, as they could reproduce annually as in their IWP distribution (Peverell 2009), or biennially as in the San Juan-Colorado basin (Thorson 1976), this crucial aspect of which there is no available information, could also have important consequences for their detection, as young-of-the-year and pupping females could be absent from pupping grounds during some years, further decreasing the chances of detecting an already rare species.

The conservation status of *P. pristis*, together with differences in abundance throughout their distribution, and the amount of sawfish-oriented research in those areas, have resulted in considerable differences in the biological data available between subpopulations (Kyne et al. 2021). For example, there are no published genetic studies from any of the *P. pristis* subpopulations except from the IWP subpopulation (e.g. Phillips et al. 2011; Feutry et al. 2015). “Lifeboat” sawfish populations (viable sawfish populations where species and their habitats are relatively well protected) from the northern part of Australia (Fordham et al. 2018) could be considered one of the few current sources of genetic material to be studied. However, our findings together with recent records in the San Juan-Colorado River seem to indicate a promising source to help elucidate aspects of population genetics within the WA subpopulation.

The extensive work carried by Faria et al (2013), established very low genetic differences between *P. pristis* subpopulations, however, their WA subpopulation analysis did not include genetic material from the Lake Nicaragua system. Thorson (1982b) suggested the population from the Lake Nicaragua system to be a discretely isolated stock, based on strong lines of evidence gathered in his many years of working in the system. Considering the latter, it is possible that the inclusion of Lake Nicaragua System samples in the analyses carried by Faria et al (2013), could have shown isolation from the Lake Nicaragua system stock, resulting in population structure within the WA subpopulation. The analyses of DNA extracted from this study’s eDNA samples revealed small genetic differences between the sequences from the San Juan-Colorado River, and the Australia – Papua New Guinea group (unpublished data). These sequences are of little use on their own, yet they are important to conduct future population analysis, and improve the understanding of the genetic relationships in and between the subpopulations of the most wide-ranging of sawfish species (Faria et al. 2013; Kyne et al. 2021).

Despite our ecological considerations regarding seasonality, *P. pristis* is after all, a critically endangered species that occurs in low numbers, and in some areas, their abundance is likely too low to be detected unless sampling occurs at the right place and at the right time. Moreover, even though the primers and probes used in this study, were designed based on DNA sequences of *P. pristis* from the IWP subpopulation (Cooper et al. 2021), they were able to match and amplify eDNA from *P. pristis* of the San Juan-Colorado River, a partially isolated stock from the WA subpopulation, and that presents genetic differences with the IWP subpopulation, as shown in the obtained sequences. Therefore, it is most likely that that our sampling effort of the EP subpopulation in the Costa Rican Pacific coast would have detected the species if present in the ecosystems at the time of our sampling. Detecting *P. pristis* eDNA in the San Juan-Colorado River is an ambivalent task. The sampling technique has to be sensitive enough to detect eDNA that occurs in low numbers in fast flowing sediment-ridden waters, however, as this specific stock spends most of its time in the Lake Nicaragua system, the sampling is not affected by seasonal movements of the species, allowing for low abundance, but continuous eDNA sources. On the other hand, eDNA sampling in the Costa Rican EP will not only be affected by the extreme rarity of the species, but also by their suggested marked seasonality. Even if sampling is carried late in the wet season to target pupping females in rivers and/or estuaries, there is a tradeoff between maximizing the chances of detection and the increased eDNA dilution, stochastic mixing, turbidity and PCR inhibitors caused by the increased flow of the rainy season (Stoeckle et al. 2017; Le Port et al. 2019). However, the identification of a riverine or estuarine nursery in the area could greatly improve the success rate of future eDNA strategies. Several promising remote sites are still to be explored in the Costa Rican EP, such as various rivers, lagoons and estuaries in the Corcovado National Park and the upper reaches of the Sierpe River in the TSNW in the south Pacific region. The lack of detections from our massive effort in the Costa Rican Pacific coast in areas that presented recent records, continues to prove the EP subpopulation of *P. pristis* as one of the least understood.

P. pristis eDNA detections and recent records along the San Juan-Colorado River, open the possibility for the species to be regaining its mobility and, therefore, connectivity within the Lake Nicaragua system and its tributaries (e.g. Frío, San Carlos, Sarapiquí) (Vincenzi & Camacho 1974; Bussing 2002). To our knowledge, there are no current research efforts for sawfish in Nicaragua, yet in 2017 and 2021, there were two reports of fishermen catching small (<1 m) *P. pristis* with gill nets inside the Lake and in the Pacific coast, respectively (A. Tisseaux pers. comm. 2021). Several threats occur along the Lake Nicaragua system, with the immediate threats being the finfish net fisheries from Lake Nicaragua and Barra del Colorado. Although the species is currently protected by both Costa Rican and Nicaraguan legislation, Nicaragua’s current regulations (MARENA 2019) protect sawfish species only within Lake Nicaragua and along the San Juan River. Although it seems *P. pristis* is starting to recover in the Lake Nicaragua system, even low fishing pressure could have a large impact on their population (Kyne et al. 2013).

Although the overall detection rate of *P. pristis* throughout the entire country was low, we were able to identify potential hotspots for the species in the San Juan-Colorado River system. Unfortunately, the use of eDNA was unsuccessful in detecting *P. pectinata*. Samples from all but two regions of the country (northern region and Northern Caribbean region) provided no detections for either sawfish species, despite that in some of these regions there has been multiple confirmed records since 2016, mainly from *P. pristis* (Valerio-Vargas & Espinoza 2019). Despite the historical presence of *P. pectinata* in Costa Rican waters (Thorson 1982b; Valerio-Vargas & Espinoza 2019), the last confirmed record of the species occurred in the southern Caribbean region in 2002 (unpublished data). However, the subtle differences between sawfish species may limit fishers' ability to distinguish them (Hossain et al. 2015), opening the possibility that some of the records from the Caribbean coast of Costa Rica could have been misidentified. Following a reduction of ≥95% of their population and 81% of their geographic range size (Dulvy et al. 2016), it is likely that *P. pectinata* may now be restricted to their core distribution range in the southern east coast of the USA and The Bahamas (Brame et al. 2019), or found in low numbers throughout Latin America, thus limiting our ability to detect the species with eDNA. There are few records of the *P. pectinata* in Belize and Honduras (Dulvy et al. 2016), yet detail information of the distribution and abundance of this species in Central America is still lacking. Belize and Honduras have some of the highest mangrove cover along the Central

American Caribbean coastline (Spalding et al. 1997), which are known to be critical habitats as nursery grounds for *P. pectinata* (Simpfendorfer 2006; Simpfendorfer et al. 2011; Guttridge et al. 2015). There are only two relatively small and isolated mangrove forests in the Caribbean of Costa Rica (Córdoba-Muñoz et al. 1998), one is located at Moín (central Caribbean), which is heavily impacted by coastal development and pollution, while the other is located in Laguna Gandoca Wildlife Refuge (southern Caribbean) (Cortés & Wehrtmann 2009). Even if *P. pectinata* was historically present in Costa Rican waters, it was far less abundant than *P. pristis* in the 1960's-70's, when Thorson (1982b) recorded hundreds of *P. pristis* and only 16 individuals of *P. pectinata* in 14 years of sampling. The latter were caught at Barra del Colorado and Boca Samay, where no mangroves are present. Moreover, to our knowledge, there are no reports of juvenile *P. pectinata* in Costa Rican waters. Given their migratory behavior and the relatively low number of confirmed records of *P. pectinata* in Costa Rican waters, it is also possible that some individuals may be potential migrants from other Central American countries (e.g. Belize and Honduras) (Brame et al. 2019).

Urgent cooperation research is needed to evaluate *P. pristis* population status in the entire Lake Nicaragua system, although Nicaragua's political instability and the global pandemic situation have hindered cooperation and research efforts. However, a more natural or ecosystem, rather than a political or synthetic approach could be successful in generating appropriate management and conservation measures for the species, as *P. pristis* is known to use and move across international borders (Thorson 1982b). A similar approach has been adopted by the governments of Honduras, Guatemala, Belize and Mexico to manage the Mesoamerican Caribbean Reef (Kramer & Kramer 2002). Such approach could allow for consensus management between Costa Rica and Nicaragua, in order to secure sawfish populations, by decreasing their threats and allow for a stable recovery of a historical core population of the WA subpopulation of *P. pristis* (Fernandez-Carvalho et al. 2014).

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6. Supporting Information

Appendix S1: *P. pristis* mitochondrial single nucleotide substitution (T to C) (light blue) observed in eDNA sequenced from the San Juan-Colorado River samples.

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                            10         20         30         40         50         60         70         80         90
Consensus          GGAGCCTGTT CTATAACCGA TAATCCCCGT TAAACCTCAC CACTTCTTGC CATCAACCGC CTATATACCG CCGTCGTCAG CTCACCCCAT
Ppristis220343     GGAGCCTGTT CTATAACCGA TAATCCCCGT TAAACCTCAC CACTTCTTGC CATCAACCGC CTATATACCG CCGTCGTCAG CTCACCCCAT
Ppristis           GGAGCCTGTT CTATAACCGA TAATCCCCGT TAAACCTCAC CACTTCTTGC CATCAACCGC CTATATACCG CCGTCGTCAG CTCACCCCAT
Ppristis100036     GGAGCCTGTT CTATAACCGA TAATCCCCGT TAAACCTCAC CACTTCTTGC CATCAACCGC CTATATACCG CCGTCGTCAG CTCACCCCAT
Ppristis210303     GGAGCCTGTT CTATAACCGA TAATCCCCGT TAAACCTCAC CACTTCTTGC CATCAACCGC CTATATACCG CCGTCGTCAG CTCACCCCAT
Ppristis220360     GGAGCCTGTT CTATAACCGA TAATCCCCGT TAAACCTCAC CACTTCTTGC CATCAACCGC CTATATACCG CCGTCGTCAG CTCACCCCAT
Ppristis220382     GGAGCCTGTT CTATAACCGA TAATCCCCGT TAAACCTCAC CACTTCTTGC CATCAACCGC CTATATACCG CCGTCGTCAG CTCACCCCAT
P. pristis220382   ---------- ---------- ---------- ----CCTCAC CACTTCTTGC CATCAACCGC CTATATACCG CCGTCGTCAG CTCACCCCAT
eDNA_Ppristis1     ---------- ---------- -------CGT TAAACCTCAC CACTTCTTGC CATCAACCGC CTATATACCG CCGTCGTCAG CTCACCCCAT
eDNA_Ppristis2     ---------- ---------- ------CCGT TAAACCTCAC CACTTCTTGC CATCAACCGC CTATATACCG CCGTCGTCAG CTCACCCCAT
eDNA_Ppristis3     ---------- ---------- ---------- ---ACCTCAC CACTTCTTGC CATCAACCGC CTATATACCG CCGTCGTCAG CTCACCCCAT
eDNA_Ppristis4     ---------- ---------- ---------- --AAACTCAC CACTTCTTGC CATCAACCGC CTATATACCG CCGTCGTCAG CTCACCCCAT
eDNA_Ppristis5     ---------- ---------- --------GT TAAACCTCAC CACTTCTTGC CATCAACCGC CTATATACCG CCGTCGTCAG CTCACCCCAT
eDNA_Ppristis6     ---------- ---------- ---------- ---ACCTCAC CACTTCTTGC CATCAACCGC CTATATACCG CCGTCGTCAG CTCACCCCAT
eDNA_Ppristis7     ---------- ---------- ---------- --AACCTCAC CACTTCTTGC CATCAACCGC CTATATACCG CCGTCGTCAG CTCACCCCAT
eDNA_Ppristis8     ---------- ---------- --------GT TAAACCTCAC CACTTCTTGC CATCAACCGC CTATATACCG CCGTCGTCAG CTCACCCCAT

                           100        110        120        130        140        150        160        170
Consensus          GAGGGAACAA AAGTAAGCAA AATGAACTAA CCTTCAACAC GTCAGGTCGA GGTGTAGCGA ATGAAGTGGA AAGAAATGGG CTACATTTT
Ppristis220343     GAGGGAACAA AAGTAAGCAA AATGAACTAA CCTTCAATAC GTCAGGTCGA GGTGTAGCGA ATGAAGTGGA AAGAAATGGG CTACATTTT
Ppristis           GAGGGAACAA AAGTAAGCAA AATGAACTAA CCTTCAATAC GTCAGGTCGA GGTGTAGCGA ATGAAGTGGA AAGAAATGGG CTACATTTT
Ppristis100036     GAGGGAACAA AAGTAAGCAA AATGAACTAA CCTTCAATAC GTCAGGTCGA GGTGTAGCGA ATGAAGTGGA AAGAAATGGG CTACATTTT
Ppristis210303     GAGGGAACAA AAGTAAGCAA AATGAACTAA CCTTCAATAC GTCAGGTCGA GGTGTAGCGA ATGAAGTGGA AAGAAATGGG CTACATTTT
Ppristis220360     GAGGGAACAA AAGTAAGCAA AATGAACTAA CCTTCAATAC GTCAGGTCGA GGTGTAGCGA ATGAAGTGGA AAGAAATGGG CTACATTTT
Ppristis220382     GAGGGAACAA AAGTAAGCAA AATGAACTAA CCTTCAATAC GTCAGGTCGA GGTGTAGCGA ATGAAGTGGA AAGAAATGGG CTACATTTT
P. pristis220382   GAGGGAACAA AAGTAAGCAA AATGAACTAA CCTTCAACAC GTCAGGTCGA GGTGTAGCGA ATGAAGT--- ---------- ---------
eDNA_Ppristis1     GAGGGAACAA AAGTAAGCAA AATGAACTAA CCTTCAACAC GTCAGGTCGA GGTGTAGCGA ATGAAGTGGA AA-------- ---------
eDNA_Ppristis2     GAGGGAACAA AAGTAAGCAA AATGAACTAA CCTTCAACAC GTCAGGTCGA GGTGTAGCGA ATGAAGTGGA AAGAAATGGG ---------
eDNA_Ppristis3     GAGGGAACAA AAGTAAGCAA AATGAACTAA CCTTCAACAC GTCAGGTCGA GGTGTAGCGA ATGAAGTGGA AAGAAA---- ---------
eDNA_Ppristis4     GAGGGAACAA AAGTAAGCAA AATGAACTAA CCTTCAACAC GTCAGGTCGA GGTGTAGCGA ATGAAGTGGA AA-------- ---------
eDNA_Ppristis5     GAGGGAACAA AAGTAAGCAA AATGAACTAA CCTTCAACAC GTCAGGTCGA GGTGTAGCGA ATGAAGTGGA AAGAA----- ---------
eDNA_Ppristis6     GAGGGAACAA AAGTAAGCAA AATGAACTAA CCTTCAACAC GTCAGGTCGA GGTGTAGCGA ATGAAGTGGA AAGAAAT--- ---------
eDNA_Ppristis7     GAGGGAACAA AAGTAAGCAA AATGAACTAA CCTTCAACAC GTCAGGTCGA GGTGTAGCGA ATGAAGTGGA AAGAAAT--- ---------
eDNA_Ppristis8     GAGGGAACAA AAGTAAGCAA AATGAACTAA CCTTCAACAC GTCAGGTCGA GGTGTAGCGA ATGAAGT--- ---------- ---------
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CONCLUSIONES

En esta tesis se logró: (1) determinar la distribución espacial y temporal del pez sierra de dientes grandes *Pristis pristis* en Costa Rica, (2) identificar el Humedal Nacional Térraba-Sierpe (Pacífico Sur) y el río San Juan-Colorado (región Norte y Caribe Norte) como zonas de importancia o “hotspots” para la conservación de la especie en el país, (3) identificar las principales amenazas que afectan a la sobrevivencia de la especie en distintas zonas geográficas, y (4) evaluar el uso del eDNA para la detección de *P. pristis* en ecosistemas costeros y ribereños de la costa Pacífica (norte, centro y sur), Zona Norte y Caribe (norte y sur) de Costa Rica. Además, este trabajo colectó información acerca de la distribución del pez sierra de dientes pequeños *P. pectinata*, y también realizó esfuerzos para su detección con eDNA. Todo este trabajo estuvo fuertemente ligado a la divulgación de resultados y a una campaña nacional de conservación que logró asegurar la protección de ambas especies en aguas nacionales mediante un decreto del Instituto Nacional de Pesca y Acuicultura (INCOPESCA) en noviembre de 2017.

P. pristis fue históricamente una especie ampliamente distribuida en Costa Rica, e inclusive común en ciertas zonas del país como el Golfo de Nicoya, el Humedal Nacional Térraba-Sierpe y la Zona Norte, también fue reportado como abundante en Barra del Colorado. Por otro lado, *P. pectinata* fue mucho menos abundante en el país, con solo dos registros constatados en ambientes costeros del Caribe. La considerable disminución en la cantidad de reportes de *P. pristis*, y zonas en las que fue reportado, indican una marcada disminución en el rango de ocurrencia de la especie en Costa Rica. Los registros más recientes obtenidos mediante las entrevistas fueron en el Humedal Nacional Térraba-Sierpe y la Zona Norte, sugiriendo a ambas zonas como puntos calientes para la especie, y destacando la necesidad de realizar investigación en ellas.

La amenaza más común fue la pesca, viéndose reflejado en que una vasta mayoría de los encuentros con *P. pristis* se dieron durante actividad de pesca, aunque la degradación y destrucción de su hábitat también fue importante. Distintos tipos de pesca representan amenazas en diferentes tipos de ecosistemas. La pesca con redes capturó muchos de los animales en la zona costera, mientras que la pesca con anzuelo capturó más organismos en ríos y esteros. La pesca con arpón desde bote (a modo de jabalina) es una amenaza única de la zona norte, en donde se sigue utilizando para la captura de sábalo real (*Megalops atlanticus*), y en ocasiones se capturan especímenes de gran tamaño de *P. pristis*, en apariencia, como pesca incidental.

La pesca es, además, la forma de obtener acceso a productos o partes de la especie, en particular a su rostro en forma de sierra, así como su carne y aletas. Su rostro era altamente valorado como adorno y representaba una parte importante del comercio alrededor de la especie. El mercado de sus dientes rostrales o de su sierra para la extracción de los dientes rostrales, con el fin de utilizarlos para la fabricación de espuelas para peleas de gallos, es una situación conocida en otras partes de Latinoamérica, pero nunca antes reportada para Costa Rica. Este trabajo reporta esta actividad tanto de forma histórica como actual, ya que aún se realiza en Costa Rica, formando parte de una red internacional del tráfico de partes de la especie. Esta amenaza pone aún más presión sobre las ya de por sí devastadas poblaciones de *P. pristis* y requiere de acciones de cooperación entre actores clave.

El eDNA fue una herramienta muy útil para ampliar el conocimiento generado mediante las entrevistas, y así tener una visión más completa de la distribución actual de *P. pristis*. Las detecciones de eDNA de *P. pristis* representan los primeros registros directos de un esfuerzo dirigido para encontrar la especie en Costa Rica desde los estudios del Dr. Thomas Thorson, hace 40 años. La técnica de eDNA utilizada es lo suficientemente sensible para detectar la especie en ambientes lóticos y es capaz de lidiar con altas cantidades de sedimento suspendido, demostrando su gran potencial para la conservación de especies acuáticas en peligro de extinción.

Las detecciones de *P. pristis* en la Zona Norte y Caribe norte apoyan la interpretación de la Zona Norte como punto caliente a partir de la información de las entrevistas e indican que todo el río San Juan-Colorado es de altísima importancia para la especie. Los registros de capturas durante 2020-2021 en el río San Juan-

Colorado sumado a los resultados de eDNA, indican una población viable y reproductiva. La ausencia de detecciones de *P. pristis* en los sitios del Caribe sur está ligada a una aparente baja abundancia de la especie en la zona, sumado a una serie de condiciones propias de los ecosistemas muestreados y al colapso de sus poblaciones en los 1980´s.

La total ausencia de detecciones en toda la costa Pacífica puede explicarse por múltiples causas. Enormes diferencias ecológicas entre la cuenca del río San Juan-Colorado y los ecosistemas de la costa Pacífica de Costa Rica parecen jugar un papel importante en el comportamiento y uso de hábitats de *P. pristis*. Similitudes en los escenarios de precipitación entre la costa Pacífica de Costa Rica y la parte norte de Australia sugieren un comportamiento similar, con una marcada estacionalidad en el uso de hábitats en diferentes etapas de su vida. El déficit de lluvia durante el periodo de muestreo pudo haber afectado el comportamiento de *P. pristis*, manteniéndolo alejado de los sitios de muestreo y por ello ausente en las muestras. La información ecológica disponible sugiere que la toma de muestras de eDNA en ecosistemas de la costa Pacífica de Costa Rica va a diferir en la etapa de vida de los organismos meta, según se tomen en ríos, esteros, aguas costeras o el mar abierto. La detección de eDNA de hembras reproductivas y de neonatos en zonas de alumbramiento va a depender de la periodicidad reproductiva de la subpoblación, sea esta anual o de año por medio.

P. pristis es una especie en peligro crítico de extinción con números que pueden ser demasiado bajos para ser detectados en algunas áreas, a menos que se tome la muestra en el momento y lugar adecuado. El muestreo de eDNA de la especie en el río San Juan-Colorado no es afectado por movimientos estacionales, mientras que en la costa Pacífica de Costa Rica va ser afectado por su posible estacionalidad y rareza extrema. La falta de detecciones de eDNA en la costa Pacífica del país sigue demostrando lo poco comprendida que es la subpoblación del Pacífico Oriental de la especie.

La protección legal otorgada por el INCOPESCA en noviembre de 2017 a ambas especies de pez sierra (*P. pristis* y *P. pectinata*) mediante el decreto ejecutivo

AJDIP/316-2017, es uno de los principales logros de este trabajo y convierte a Costa Rica en el país número 17 en otorgar algún grado de protección legal a los peces sierra. Sin embargo, es tan solo el primer paso para salvaguardar el futuro de las especies de peces sierra en el país, y es necesario seguir trabajando en la concientización para lograr una mayor comprensión del problema por parte de la ciudadanía y aplicación de las leyes por parte de las autoridades. La presencia de las autoridades en las zonas remotas en las que habita la especie es mínima o nula, por lo que la educación y concientización son primordiales para la conservación de la especie.

La protección legal sienta las bases para la urgida cooperación internacional con Nicaragua en un escenario de aparente recuperación de la especie. Se necesitan estudios para evaluar su estado poblacional en el sistema Lago de Nicaragua. *P. pristis* habita y se mueve a través de aguas continentales internacionales, por lo que su adecuada protección requiere de un enfoque a nivel del ecosistema para disminuir sus amenazas y permitir la recuperación de sus poblaciones.

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