Alma Mater Studiorum – Università di Bologna

DOTTORATO DI RICERCA IN
BENI CULTURALI E AMBIENTALI

Ciclo 34

Settore Concorsuale: 05/B1 Zoologia e antropologia

Settore Scientifico Disciplinare: BIO/05 Zoologia

TOWARDS THE CONSERVATION OF VULNERABLE MARINE LARGE PREDATORS: MORPHOMETRIC, MOLECULAR AND MICROCHEMICAL VARIATION OF HISTORICAL SAWFISH ROSTRA FROM MEDITERRANEAN COLLECTIONS

Presentata da: Valentina Crobe

Coordinatore Dottorato

Roberto Pasini

Supervisore

Fausto Tinti

Co-Supervisori

Alessia Cariani

Elisabetta Cilli

Esame finale anno 2022

Nothing in life is to be feared, it is only to be understood.
Now is the time to understand more, so that we may fear less.

Maria Salomea Skłodowska (M. Curie)

Thesis Abstract

Sawfishes (Chondrichthyes, Pristidae) are considered one of the most endangered families among elasmobranchs, resulting extinct in many coastal areas around the world. A growing concern in conserving these iconic animals has been significant worldwide, including efforts to gather robust anecdotal information on historic and recent changes in species composition/range.

In this study, we have implemented an integrative approach to characterize the species diversity and the abundance of historical rostra of sawfishes from museums and private collections of the Mediterranean area. To solve some morphological ambiguities caused by the similarity among species and their rarity, the identification at the species level of 172 dried rostra was carried out through the integration of both traditional and geometric morphometric techniques with molecular tools, allowing the assessment of a robust methodical approach to discriminate species. The molecular taxonomic identification at the species level was obtained through DNA extraction from rostra and sequencing of small fragments (ca. 320-350 bp) of two mitochondrial markers (COI and NADH2). In addition, we analysed 35 rostral teeth to clarify the past distribution of sawfish species considering the isotopic composition of oxygen and carbon. The morphometric, molecular, and geographical characterization of samples was accompanied by the preliminary evaluation of growth structures and the inspection of the strontium isotope composition in two teeth to unravel movement patterns of individuals across different salinities of water.

Results were integrated with currently available data from public repositories and these analyses showed that the historical specimens belonged to four nominal species: *Pristis zijsron* (81), *Anoxypristis cuspidata* (39), *P. pristis* (30), and *P. pectinata* (22). An identification error of 5.41% emerged in the morphological distinction of rostra between juvenile individuals of *P. pectinata* and *P. zijsron*. The new approach of carbon and oxygen isotopes, implemented for the first time in these taxa, permitted the identification of the high-probability habitat preferences of these benthopelagic elasmobranchs in about 50% of the analysed specimens. Fifteen individual rostra have been assigned to a given geographical area of origin with high probability. Among them, 12 individuals were precisely assigned to the Mediterranean, the Red Sea and Persian Gulf (11 individuals of *P. zijsron* and one *P. pristis*). The geographical assignment of other 20 individuals was not univocally achieved by isotope analysis since two to four isoscapes were matched by the individual $\delta^{18}O$ composition.

Using this multidisciplinary approach, we successfully assigned the numerous museum rostra with lacking data to a given species and identified their candidate geographical origin, retrieving novel information and data for understanding the species distribution and ecology of past, sometimes locally/regionally extinct sawfish faunas (e.g., as it is the one of the Mediterranean Sea).

Research objectives

The unprecedented global reduction of sawfish (Chondrichthyes, Pristidae) led the scientific community to address huge and widespread research efforts on the recovery of remaining populations in the world. Immediate actions are required to better determine the status of the species in poorly studied regions and to initiate biological studies to provide the data needed to establish management and restoration plans.

In this perspective, this PhD project was conceived for contributing to the conservation of this iconic elasmobranch family and help the reconstruction of their species diversity, distribution, and movement. Specifically, the project's objectives were to: 1) comprehensively understand the current state of knowledge on sawfish at the global scale, identifying future research priorities needed for better assisting their conservation; 2) characterize at the species level all rostra specimens conserved in museums and private collections of the Mediterranean area; 3) examine the applicability of the isotopic composition of oxygen and carbon in rostral teeth to determine the geographical origin of specimens and to clarify their distribution; 4) improve methods of sectioning, imaging and recording strontium isotope in rostral teeth to assess their potential as a chemical tracer for describing movement pattern.

With the aim of delineating future research priorities for the conservation of sawfish, key-existing information about their biology, ecology and major threats was reviewed in **Chapter 1**. In this Chapter current mismatches between their biology/ecology and the management strategies were widely discussed, pointing out those factors that are negatively affecting the development of effective conservation strategies.

According to the importance of characterizing sawfish collections of the Mediterranean area, in **Chapter 2** wide bibliographic research was compiled to find out all the museums and private collections which possessed entire sawfish or rostra specimens. Specifically, 172 dry museum rostra or whole taxidermized individuals conserved in 27 museum/private collections of the Mediterranean area were identified with the dual approach that combines morphometric analysis of rostrum and molecular analysis optimized for ancient DNA. The results of this research represented an example of the utility of morphological and molecular tools for the conservation of these organisms, providing a systematic means to overcome the difficulties imposed by the absence of diagnostic morphological traits.

Chapter 3 was focused on oxygen and carbon isotopes characterisation in sawfish teeth and their potential use for the determination of specimens' provenance. In this chapter, the development of the isoscape analysis technique in 35 rostral teeth was discussed.

Finally, the preliminary analyses of sectioning and imaging of sawfish rostral teeth were tested and assessed in **Chapter 4**. The image of two rostral teeth sections allowed to preliminary assess the presence of the external thin enamel layer. They were analysed by laser ablation–inductively coupled plasma mass spectrometry (LA-ICPMS) of $^{87}Sr/^{86}Sr$ isotope ratio, for the potential use as a chemical record of movement through different salinities.

Table of contents

Chapter 1

General introduction

1.1 Biology, ecology, and distribution of sawfishes

Sawfishes (Family Pristidae, Chondrichthyes) are large body size batoids (up to ~7 m) with a characteristic rostrum bearing lateral tooth-like denticles (Last & Stevens 2009). The family includes two genera, *Anoxypristis* and *Pristis*. There is currently a single recognized species of *Anoxypristis*, *A. cuspidata* (Latham, 1794) and four extant *Pristis* species, *P. clavata* Garman 1906, *P. pectinata* Latham 1794, *P. pristis* Latham 1794 and *P. zijsron* Bleeker 1851 (Faria et al., 2013). For a long time, the Pristidae family was considered as one of the most taxonomically chaotic groups within the batoids, due to their conservative morphology (Faria et al., 2013): until 2013, the genus *Pristis* comprised seven species and were partitioned into the smalltooth (i.e., *P. clavata*, *P. pectinata* and *P. zijsron*) and the largetooth groups (i.e., *P. microdon*, *P. perotteti*, and *P. pristis*).

In general, sawfishes are benthopelagic and inhabit shallow coastal waters (<10 m), including estuaries and rivers of the tropics and subtropics (Dulvy et al., 2016), though to occur to a maximum depth of 122m (Poulakis & Seitz, 2004; Wueringer, Squire, & Collin, 2009). A strong relationship with shallow-water habitats such as mangrove shorelines and muddy or sandy bottoms has been documented (Poulakis & Seitz, 2004; Thorburn et al., 2008; Whitty et al., 2009; Morgan et al., 2015; Morgan et al., 2017).

The life history of sawfishes is typical of many coastal elasmobranchs, as they exhibit a low growth rate, late maturity (between three and nine years, depending on the species), low fecundity (from one to 20 pups for each litter), large size at birth and high longevity (Peverell, 2005; García, Lucifora, & Myers, 2008; Thorburn et al., 2008; Simpfendorfer & Kyne, 2009). They are characterised by lecithotrophic viviparity or ovoviviparity, retaining embryos until birth, once development is complete (Dulvy & Reynolds, 1997). These parameters result in a low intrinsic rate of population growth, highlighting susceptibility to population depletion (Simpfendorfer, 2000).

As other elasmobranchs, they show ontogenetic shifts in habitat use, where juveniles dwell shallow and enclosed coastal or riverine habitats that offer high productivity and protection from predators as nurseries, before moving to deeper marine habitats as adults (Heupel, Carlson, & Simpfendorfer, 2007). Thus, inshore and estuarine waters are critical habitats for juveniles and pupping females (Poulakis & Seitz, 2004; Peverell, 2005; Last et al., 2009; Whitty et al., 2009, 2017; Poulakis et al., 2013; Carlson et al., 2014). In detail, the life history of *P. pristis* is unique in comparison to the other sawfishes, in that females give birth near river mouths and pups migrate upriver to freshwater habitats, where they remain until sexual maturity (Whitty et al., 2009; Kyne et al., 2021). Adults of *P. clavata* are marine/estuarine and juveniles utilize inshore estuaries and mangrove areas as nurseries (Thorburn et al., 2008). This species has been known to penetrate rivers, but only as far as the tidal limit (Morgan et al., 2021). Similarly, *P. pectinata* exhibits ontogenetic habitat shifts (Grubbs, 2010) with small juveniles exhibiting high site fidelity and spending all their time in the shallow waters along mangrove shorelines, while larger juveniles and adults are known to use deeper water within and beyond estuaries (Poulakis et al., 2013; Hollensead et al., 2016, 2018; Graham et al., 2021). In *P. zijsron*, home-range size increases with growth and development (Morgan et al., 2015; 2017). Neonates generally stay close to shallow water (<0.5 m depth) for at least their first few months of life and become increasingly mobile with increasing body size (Morgan et al., 2017). Juveniles show nursery site fidelity and are more likely to be recorded outside of the estuaries (Peverell, 2005). In this species, low-salinity waters do not provide favourable conditions (Morgan et al. 2017). Finally, less is known about life history traits of *A. cuspidata*, which reach maturity comparatively earlier, at approximately 3 years, and are found in correspondence of estuaries, bays, and river mouths from the shallows to 40 m of depth (Last and Stevens, 2009).

Sawfishes were once widely distributed and typically found in tropical and sub-tropical locations (Dulvy et al., 2016). More precisely, *P. pristis* has the widest range distribution with four distinct subpopulations from Eastern Atlantic, Western Atlantic, Eastern Pacific and Indo-West Pacific (Faria et al., 2013). The historic distribution of the largetooth sawfish (Figure 1, A) is somewhat uncertain

and largely unknown because of poor records and questionable identifications associated with the difficulties in distinguishing among *Pristis* sawfishes and presumed localized extinctions in many locations (Last & Stevens, 2009; White & Kyne, 2010; Morgan et al., 2011; Ferretti et al., 2016). *Pristis pectinata* is reported from West and East Atlantic subtropical waters (Faria et al., 2013) (Figure 1, B). The species was historically distributed in coastal habitats of the Gulf of Mexico and along the east coast of the United States but currently, the only remaining viable population is centred in southwest Florida (Norton et al., 2012; Dulvy et al., 2016; Graham et al., 2021). *Pristis zijsron* and *A. cuspidata* spread from the Red Sea to the Australian coasts (Dulvy et al., 2016) (Figure 1, C and E, respectively). Once widely distributed along the Indian Ocean coasts, with catch records from South Africa, India, Southeast Asia, and northern Australia (Peverell, 2005; Last & Stevens, 2009), their current global distribution is mostly uncertain (Dulvy et al., 2016). Globally, the historical distribution of *P. clavata* is largely unknown (Figure 1, D) and it is believed to be restricted to northern Australia (Thorburn et al., 2008; Morgan et al., 2021).

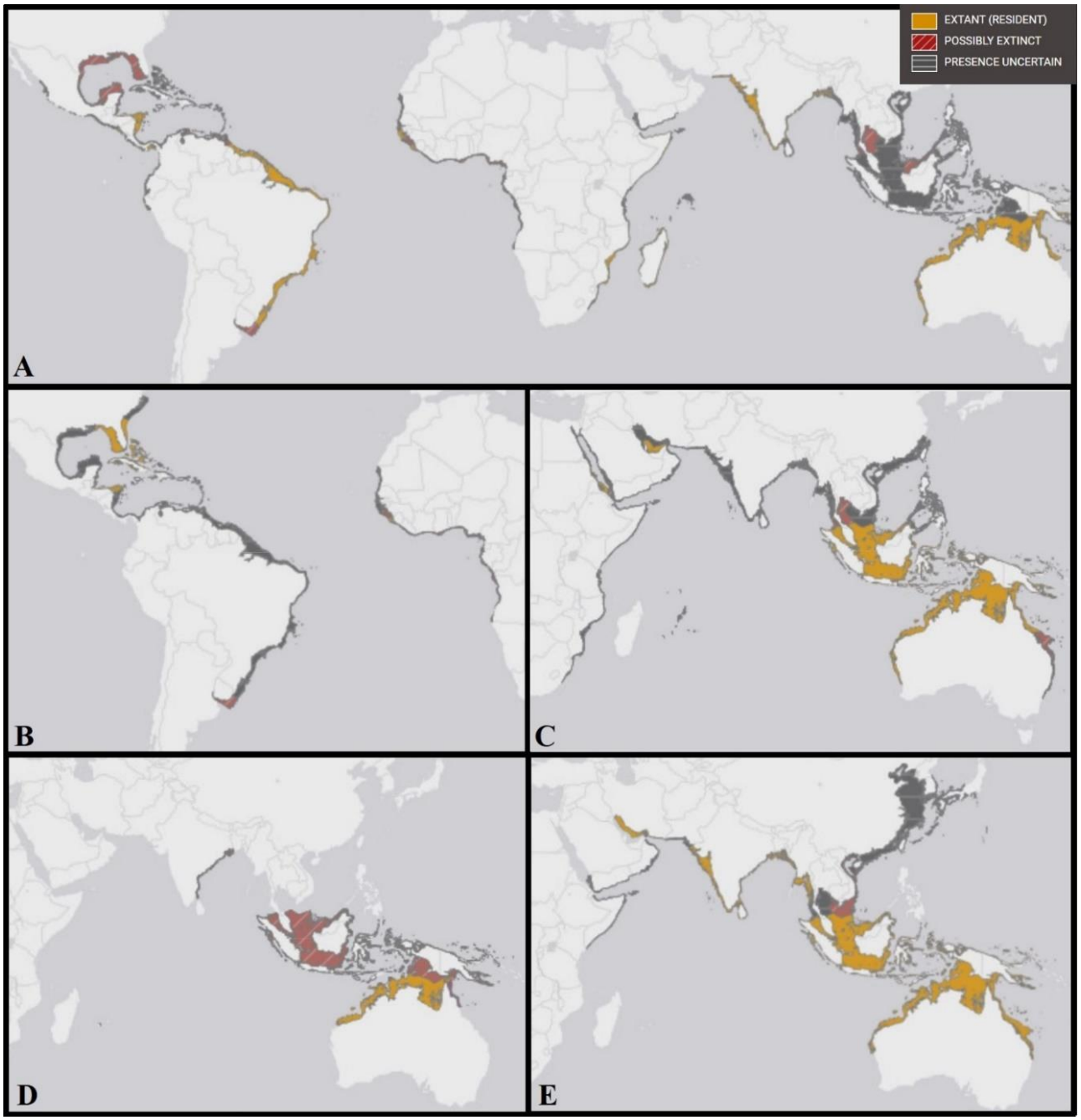

Figure 1 Current, past and uncertain distribution of the five sawfish species. A) *P. pristis*; B) *P. pectinata*; C) *P. zijsron*; D) *P. clavata* and E) *A. cuspidata*. Maps were taken from the last assessments of IUCN Red List of Threatened Species (Simpfendorfer, 2013; D'Anastas et al., 2013; Carlson et al., 2013; Kyne et al., 2013; 2021).

Sawfish species share a high similarity of body morphology; however, they could be distinguished for some diagnostic features as the origin of the first dorsal fin with respect to the origin of the pelvic fin, the morphology of the caudal fin and the structure of the rostrum. In detail, *A. cuspidata* showed the origin of the first dorsal fin slightly posterior to that of the pelvic fins, while in *P. pristis* is anterior (Compagno & Last, 1999). In the other three species, the first dorsal fin origins inline or just posterior to the pelvic fins (Compagno & Last, 1999). Moreover, they could be discriminated by the lower caudal fin lobe: *A. cuspidata* and *P. pristis* have the lobe defined, unlike the other three species (Wueringer, Squire, & Collin, 2009). The variation of morphometric characters of the rostrum has been demonstrated to discriminate among sawfish species. Some measures frequently used are total rostrum length, standard rostrum length, standard rostrum width, rostrum tip width, proximal tooth gap, distal tooth gap, the distance between the proximal teeth and distance between distal teeth (Whitty et al., 2014; Seitz & Hoover, 2017; Chapter 2 of this thesis). These measures and their ratio could discriminate between sawfish species and confirm or solve taxonomy problem for sample without identification at the species level (Faria et al., 2013; Whitty et al., 2014; Seitz & Hoover, 2017).

In this family, the saw ends with the cartilaginous cranium and possess tooth-like denticles that are externally protracted. Unlike sawshark (order Pristiophoriformes) rostral teeth, tooth-like denticles of sawfish are permanent, with continuous growth along with the life of individuals and are not replaced (Miller, 1974; Byler, 2017). The rostrum gives to sawfishes the ability to feed in the water column hitting and stunning fish prey with their lateral teeth, and then they bring the prey to the bottom. Additionally, they use the saw to stir sandy and muddy substrate once preys were perceived thanks to the Ampullae of Lorenzini (Wueringer, 2012).

The two genera differ for internal rostral anatomy. Both genera have a middle channel full of connective lax tissue that ends to the third distal tooth of the rostrum, but *Pristis* has only one pair of lateral channels instead of the two pairs as in *Anoxypristis* (Wueringer, Squire, & Collin, 2009). In both genera, the lateral channels contain ophthalmic and buccal nerves and the rostral artery (Wueringer, Squire, & Collin, 2009; Byler, 2017). Another internal anatomical difference is the position of the Ampullae of Lorenzini. In the genus *Pristis* they are located out the cartilage and are covered by connective tissue, while in *Anoxypristis* they are located between the two ophthalmic and buccal ampullae contained in the second pair of lateral channels (Wueringer, Squire, & Collin, 2009; Byler, 2017).

1.2 Anthropogenic threats and conservation

Given the low reproductive potential derived by their intrinsic biological characteristics, sawfish are particularly susceptible and cannot sustain even the slightest anthropogenic stressors (Dulvy et al., 2016; Yan et al., 2021). Consequently, they have undergone dramatic declines in range and abundance due to the combined effects of targeted catch and bycatch mortality, habitat loss and degradation (e.g., destruction of mangrove shorelines), and commercial trade of sawfish parts (e.g., highly valued rostra and fins) (Brame et al., 2019; Poulakis & Grubbs, 2019). Recently it has been estimated that sawfish species, which in the past were found in the coastal areas of 90 countries, today are presumed extinct in more than half, and in some countries more than one species has been lost (Yan et al., 2021).

Mortality resulting from artisanal, commercial, and recreational fisheries has been the primary cause of sawfish population declines worldwide (Brame et al., 2019; Poulakis & Grubbs, 2019). Their large size and the long-toothed rostrum caused sawfishes to be frequently stuck in fishing nets, especially gill nets and trawls, with high rate of mortality.

The habitat has a critical role in the extinction rate, considering that sawfishes inhabit different coastal areas at all life stages, with ontogenetic shift of habitats (see previous paragraph), which are more threatened by intense urbanization and human activities. Living in altered or fragmented habitats may cause chronic stress especially in juveniles (Prohaska et al., 2018). One of the important foundation habitats is the mangroves ecosystems, whose deforestation due to urban development (Friess & Webb, 2014) contributed to the dramatic reduction of sawfish populations (Yan et al., 2021).

For all these threatens, the International Union for Conservation of Nature (IUCN) classified three species (*P. pristis, P. zijsron, P. pectinata)* as Critically Endangered, and the remaining two species (*A. cuspidata, P. clavata*) as Endangered (Simpfendorfer, 2013; D'Anastasi, Simpfendorfer, & van Herwerden, 2013; Carlson, Wiley, & Smith, 2013; Kyne, Rigby, & Simpfendorfer, 2013; Kyne et al., 2021). International trade of sawfish is forbidden since 2007 under the Convention on International Trade in Endangered Species (CITES) of Wild Fauna and Flora (McClenachan, Cooper, & Dulvy, 2016). Although the awareness on the sawfish conservation has increased in recent years, only a few countries introduced specific laws to preserve them, such as the United States and Australia. The U.S. federal Endangered Species Act (ESA) and the Australian Environment Protection and Biodiversity

Conservation Act implemented management measures and recovery plans (Dulvy et al., 2016). The ban of fishing and trade of sawfish are effective actions to reduce or stop mortality in those countries (Fordham et al., 2018).

However, conservation actions are required to better assess the status of the species in poorly studied regions. In recent years, numerous other countries are putting efforts toward determining the conservation status of sawfish, such as Brazil (Reis-Filho et al., 2016), Guinea-Bissau (Leeney & Poncelet, 2015), countries of the Mediterranean Sea (Ferretti et al., 2016), Red Sea and Arabian Gulf countries (Jabado et al., 2017; Elhassan, 2018), Bangladesh (Haque & Das, 2019; Haque, Leeney, & Biswas, 2020) and Papua New Guinea (White et al., 2017). All research efforts must be accompanied by the enforcement of effective management actions and by fostering of fisher behavioral changes with public outreach and education programs (Kroetz et al., 2021). For instance, specimens should be handled and released properly to ensure survival when accidentally fished (Prohaska et al. 2018), instead, reports of sawfish released after the removal of the rostrum are still common (Morgan et al., 2016; Cabanillas-Torpoco et al., 2020).

Genetic studies have been proven useful in answering questions related to biology ecology, and the recovery potential of sawfish populations (Poulakis & Grubbs, 2019). Despite precipitous declines, some sawfish populations in the United Stated and Australia have shown generally high genetic diversity (Chapman et al., 2011; Phillips et al., 2016). However, some assemblages of Australian *Pristis* and *Anoxypristis* may have experienced population bottlenecks or founder effects at some time (Phillips, Fearing, & Morgan, 2017; Green et al., 2018). Otherwise, studies of eDNA were a powerful method to uncover sawfish in regions where fisheries data are scarce (Simpfendorfer et al., 2016; Lehman et al., 2020, 2022; Bonfil et al., 2021; Sani et al., 2021). The use of eDNA methods could be recommended for the early detection of their presence to help conservationists foster recovery (Poulakis & Grubbs, 2019).

1.3 Historical occurrence of Sawfish in the Mediterranean Sea

Revised taxonomy and misidentification of sawfish records have hampered sawfish conservation. These problems resulted in uncertainty in terms of distribution, life history, and population status, which have reduced the effectiveness of conservation measures (Dulvy et al., 2016). The status of Mediterranean sawfish population is illustrative of this uncertainty about sawfish geographic distribution. In their review of sawfish global population structure, Faria et al. (2013) suggested that sawfishes had never formed resident, breeding or core populations in the Mediterranean. Their presence in the basin has been debated for decades, thus considering sawfish non-resident and occasional visitors (Dulvy et al., 2016; Faria et al., 2013). Such a conclusion was based on the perception that reliable evidence of sawfish historical occurrences in the basin is lacking, and on the fact that Mediterranean water temperatures were not suitable for the occurrence of sawfish populations (Dulvy et al., 2016).

Nevertheless, the evidence supporting the Mediterranean as formerly part of the sawfish's distribution range was based on original and independent catch records, most related to juveniles or young-of-the-year, indicative of local parturition (Ferretti et al., 2016). In particular, it was documented the occurrence of two species in the basin, the largetooth sawfish (*P. pristis*) and the smalltooth sawfish (*P. pectinata*) assumed locally extinctic around 1975-1979 and 1966-1970 respectively (Ferretti et al., 2016). The study suggested that, despite apparent incongruences between the biogeography of the species and the oceanographic features of the Mediterranean Sea, sawfishes were present in the basin. Therefore, Ferretti et al. (2016) raised important questions on how the species could have occurred in this region. All the information about sawfish habitat preferences and physiological requirements (including temperature tolerance) are mainly based on few sawfish populations of Florida and northern Australia (Whitty et al., 2009; Poulakis et al., 2013; Kyne et al., 2021), and thus might not be representative of the ecological adaptations of extinct populations.

Mediterranean historical records of sawfish are known from the antiquity (Romero, 2012; Ferretti et al., 2016), unfortunately without quantitative, taxonomic, and geographic details. Numerous zoological publications from the 17th century were mostly mythological and religious (White, 2002) and sawfishes were often descripted as a mix of cetaceans, fishes and fantastic beats (Romero, 2012). During the last centuries, several scientific species catalogues were published from many sectors of

the Mediterranean: Gulf of Naples (Costa, 1857), Adriatic Sea (de Robertis, 1853), Sardinia (Casalis, 1836), Elba Island (Koestlin, 1780), Sicily (Rafinesque, 1810; Castronovo, 1872; Doderlein, 1879; Longo, 1882; Saitta, 1902), Turkia (Bilecenoglu et al., 2002), Syria (Gruvel, 1931), Algeria (Dieuzeide et al., 1953); France (Duhamel Du Monceau, 1777). Most of these catalogues reported the presence of sawfishes with details of catches or landings (see more details in Ferretti et al., 2016, Supplementary material S1). The 19th and 20th centuries were characterised by numerous records of catches and sightings reported by ichthyologists, fisher observations or other researchers (Gibert, 1913; Despott, 1919; Lozano, 1928). The first doubts on the occurrence of sawfish in the Mediterranean Sea began during the last century due to their rarity (Tortonese, 1956; Whitehead et al., 1984).

The most important historical report has been the occurrence of juvenile individuals in Syria, attributed to the species *P. pectinata*, (Gruvel, 1931). Since juveniles have never been observed to undertake major migrations such as those necessary to reach Syria from the westernmost part of the basin, these individuals could 1) constitute a local population settled in the Levantine Sea 2) be immigrants from the Red Sea. However, the only sawfish species reported from the Red Sea are *A. cuspidata* and *P. zijsron* (Faria et al., 2016). Unfortunately, the lack of specimen details led unclear whether Gruvel might have misidentified the species occurring in Syria with *P. zijsron* (Ferretti et el., 2016).

The following Chapters contribute to retrieve novel information and data for understanding the species distribution and ecology of past, sometimes locally/regionally extinct, sawfish faunas using a multidisciplinary approach that combined the species integrative taxonomy with the analysis of stable isotopes of numerous rostra of Mediterranean museums.

1.4 References

Bilecenoglu, M., Taskavak, E., Mater, S. & Kaya, M. (2002). Checklist of the marine fishes of Turkey. *Zootaxa*, 113, 1–194.

Bonfil, R., Palacios-Barreto, P., Vargas, O. U. M., Ricaño-Soriano, M., & Díaz-Jaimes, P. (2021). Detection of critically endangered marine species with dwindling populations in the wild using eDNA gives hope for sawfishes. *Mar. Biol.* **168**, 60.

Brame, A. B., Wiley, T. R., Carlson, J. K., Fordham, S. V., Grubbs, R. D., Osborne, J., Scharer, R. M., Bethea, D. M., & Poulakis, G. R. (2019). Biology, ecology, and status of the smalltooth sawfish *Pristis pectinata* in the USA. *Endanger. Species Res.* **39**, 9–23.

Byler, J. (2017). The Identification, Structure, Care, and Conservation of Sawfish Rostra (Rhinopristiformes: Pristidae). *Collect. Forum* **31**, 1–14.

Cabanillas-Torpoco, M., Castillo, D., Siccha-Ramírez, R., Forsberg, K., Purizaca, W., & Maceda, M. (2020). Occurrence of the largetooth sawfish *Pristis pristis* (Linneaus, 1758) in northern Peru. *Zootaxa* **4868**, 147–150.

Carlson, J. K., Gulak, S. J. B., Simpfendorfer, C. A., Grubbs, R. D., Romine, J. G., & Burgess, G. h. (2014). Movement patterns and habitat use of smalltooth sawfish, *Pristis pectinata*, determined using pop-up satellite archival tags. *Aquat. Conserv. Mar. Freshw. Ecosyst.* **24**, 104–117.

Carlson, J. K., Wiley, T., & Smith, K. (2013). *Pristis pectinata* (errata version published in 2019). *The IUCN Red List of Threatened Species.*

Casalis, G. (1836). Dizionario geografico strorico-statistico-commerciale negli stati di S.M. il Re di Sardegna. vol. III.

Castronovo, V. (1872). Erice oggi Monte San Giuliano in Sicilia: memorie storiche. Erice oggi Monte San Giuliano in Sicilia: memorie storiche. Lao.

Chapman, D. D., Simpfendorfer, C. A., Wiley, T. R., Poulakis, G. R., Curtis, C., Tringali, M., Carlson, J. K., & Feldheim, K. A. (2011). Genetic Diversity Despite Population Collapse in a Critically Endangered Marine Fish: The Smalltooth Sawfish (Pristis pectinata). *J. Hered.* **102**, 643–652.

Compagno, L. J. V., & Last, P. R. (1999). Family Pristidae: Sawfish. *FAO Identif. Guide Fish. Purp. Living Mar. Resour. West. Cent. Pac. FAO Rome*.

Costa, O. (1857). Fauna del Regno di Napoli ossia Enumerazione di Tutti gli Animali che Abitano le Diverse Regioni di Questo Regno e le Acque che le Bagnano: Contenente la Descrizione de Nuovi o Poco Esattamente Conosciuti. Pesci. vol. 3. Stabilimento Tipografico F. Azzolino, Napoli.

D'Anastasi, B., Simpfendorfer, C., & van Herwerden, L. (2013). *Anoxypristis cuspidata* (errata version published in 2019). *The IUCN Red List of Threatened Species.*

de Robertis, G. (1853). Del Rostro di Sega Marina (Pristis antiquorum) che Conservasi nella Reale Chiesa del Carmine Maggiore di Questa Città di Napoli in Memorie di Portento Oprato da Maria SSa. del Carmine Illustrazione Umiliata a Sua Maesta Massimiliano Giuseppe II. Re di Baviera dai Rr. Pp. Carmelitani l'anno 1853: Ex bibl. Ludovici II., Bav. Reg

Despott, G. (1919). The Ichthyology of Malta. Critien's Press, 34, Str. Reale, Valletta, Malta

Dieuzeide, R., Novella, M. & Roland, J. (1953). Catalogue des poissons de cotes algeriennes. Ia Squales, Raies, Chimeres. Bull. Sta. Aquic. Pech. Castiglione, 4, 135p.

Doderlein, P. (1879). Prodromo della fauna ittiologica della Sicilia ossia prospetto metodico delle varie specie di pesci che vennero sin ora riscontrate nei mari di Sicilia. Atti Accad. Sci. Lett. Arti Palermo, 6.

Duhamel Du Monceau, H. (1777). Trait´e G´en´eral des Pesches: et Histoire des Poissons Qu'elles Fournissent Tant Pour la Subsistance des Hommes que Pour Plusieurs Autres Usages qui ont Rapport aux Arts et au Commerce. vol. Part 2, Tome 3, Section 9. Saillant & Nyon, Paris.

Dulvy, Nicholas K., & Reynolds, J. D. (1997). Evolutionary transitions among egg–laying, live–bearing and maternal inputs in sharks and rays. *Proc. R. Soc. Lond. B Biol. Sci.* **264**, 1309–1315.

Dulvy, Nicholas K., Davidson, L. N. K., Kyne, P. M., Simpfendorfer, C. A., Harrison, L. R., Carlson, J. K., & Fordham, S. V. (2016). Ghosts of the coast: global extinction risk and conservation of sawfishes. *Aquat. Conserv. Mar. Freshw. Ecosyst.* **26**, 134–153.

Elhassan, I. S. (2018). Occurrence of the green sawfish *Pristis zijsron* in the Sudanese Red Sea with observations on reproduction. *Endanger. Species Res.* **36**, 41–47.

Everett, B. I., Cliff, G., Dudley, S. F. J., Wintner, S. P., & Elst, R. van der. (2015). Do sawfish *Pristis spp*. Represent South Africa's first local extirpation of marine elasmobranchs in the modern era? *African Journal of Marine Science*, *37*(2), 275–284.

Faria, V. V., McDavitt, M. T., Charvet, P., Wiley, T. R., Simpfendorfer, C. A., & Naylor, G. J. P. (2013). Species delineation and global population structure of Critically Endangered sawfishes (Pristidae). *Zool. J. Linn. Soc.* **167**, 136–164.

Feldheim, K. A., Fields, A. T., Chapman, D. D., Scharer, R. M., & Poulakis, G. R. (2017). Insights into reproduction and behavior of the smalltooth sawfish *Pristis pectinata*. *Endanger. Species Res.* **34**, 463–471.

Fernandez-Carvalho, J., Imhoff, J. L., Faria, V. V., Carlson, J. K., & Burgess, G. H. (2014). Status and the potential for extinction of the largetooth sawfish *Pristis pristis* in the Atlantic Ocean. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *24*(4), 478–497.

Ferretti, F., Verd, G. M., Seret, B., Šprem, J. S., & Micheli, F. (2016). Falling through the cracks: the fading history of a large iconic predator. *Fish Fish.* **17**, 875–889.

Fordham, S. V., Jabado, R., Kyne, P. M., Charvet, P., & Dulvy, N. K. (2018). Saving sawfish: Progress and priorities. *Vanc. Can. IUCN Shark Spec. Group*.

Friess, D. A., & Webb, E. L. (2014). Variability in mangrove change estimates and implications for the assessment of ecosystem service provision. *Glob. Ecol. Biogeogr.* **23**, 715–725.

García, V. B., Lucifora, L. O., & Myers, R. A. (2008). The importance of habitat and life history to extinction risk in sharks, skates, rays and chimaeras. *Proc. R. Soc. B Biol. Sci.* **275**, 83–89.

Gibert, A.M. (1913). Fauna ictiològica de Catalunya. Boletìn de la Institucìon Catalana de Historia Natural, 2° epoca, 9, 1–96

Graham, J., Kroetz, A. M., Poulakis, G. R., Scharer, R. M., Carlson, J. K., Lowerre-Barbieri, S., Morley, D., Reyier, E. A., & Grubbs, R. D. (2021). Large-scale space use of large juvenile and adult smalltooth sawfish *Pristis pectinata*: implications for management. *Endanger. Species Res.* **44**, 45–59.

Green, M. E., D'Anastasi, B. R., Hobbs, J.-P. A., Feldheim, K., McAuley, R., Peverell, S., Stapley, J., Johnson, G., Appleyard, S. A., White, W. T., Simpfendorfer, C. A., & Herwerden, L. van. (2018). Mixed-marker approach suggests maternal philopatry and sex-biased behaviours of narrow sawfish *Anoxypristis cuspidata*. *Endanger. Species Res.* **37**, 45–54.

Grubbs, R. D. (2010). Ontogenetic Shifts in Movements and Habitat Use. In *Sharks Their Relat. II*. CRC Press.

Gruvel, A. (1931). Les Etats de Syrie. Richesses marines et fluviales. Exploitation actuelle et Avenir. Soc. Edit Geogr, Marit. et Colon, Paris.

Haque, Alifa B., & Das, S. A. (2019). First confirmed record of the Critically Endangered green sawfish *Pristis zijsron* from Bangladeshi waters. *J. Fish Biol.* **94**, 200–203.

Haque, Alifa Bintha, Leeney, R. H., & Biswas, A. R. (2020). Publish, then perish? Five years on, sawfishes are still at risk in Bangladesh. *Aquat. Conserv. Mar. Freshw. Ecosyst.* **30**, 2370–2383.

Heupel, M. R., Carlson, J. K., & Simpfendorfer, C. A. (2007). Shark nursery areas: concepts, definition, characterization and assumptions. *Mar. Ecol. Prog. Ser.* **337**, 287–297.

Hollensead, L. D., Grubbs, R. D., Carlson, J. K., & Bethea, D. M. (2016). Analysis of fine-scale daily movement patterns of juvenile *Pristis pectinata* within a nursery habitat. *Aquat. Conserv. Mar. Freshw. Ecosyst.* **26**, 492–505.

Hollensead, L. D., Grubbs, R. D., Carlson, J. K., & Bethea, D. M. (2018). Assessing residency time and habitat use of juvenile smalltooth sawfish using acoustic monitoring in a nursery habitat. *Endanger. Species Res.* **37**, 119–131.

Jabado, R., Al Baharna, R., Al Ali, S., Al Suwaidi, K., Al Blooshi, A., & Al Dhaheri, S. (2017). Is this the last stand of the Critically Endangered green sawfish *Pristis zijsron* in the Arabian Gulf? *Endanger. Species Res.* **32**, 265–275.

Koestlin, C. (1780). Lettres sur l'histoire naturelle de l'isle d'Elbe. Kraus, Jean Paul, Vienne.

Kroetz, A. M., Brame, A. B., Bernanke, M., McDavitt, M. T., & Wiley, T. R. (2021). Tracking public interest and perceptions about smalltooth sawfish conservation in the USA using Instagram. *Aquat. Conserv. Mar. Freshw. Ecosyst.* **31**, 2901–2909.

Kyne, P. M., Carlson, J., & Smith, K. (2013). *Pristis pristis* (errata version published in 2019). *The IUCN Red List of Threatened Species.*

Kyne, P. M., Oetinger, M., Grant, M. I., & Feutry, P. (2021). Life history of the Critically Endangered largetooth sawfish: a compilation of data for population assessment and demographic modelling. *Endanger. Species Res.* **44**, 79–88.

Kyne, P.M., Rigby, C., & Simpfendorfer, C. (2013). *Pristis clavata* (errata version published in 2019). *The IUCN Red List of Threatened Species.*

Last, P. R. & Stevens, J. D. (2009). Sharks and rays of Australia. Second Edition. CSIRO Division of Fisheries, Melbourne, Australia

Leeney, R. H., & Poncelet, P. (2015). Using fishers' ecological knowledge to assess the status and cultural importance of sawfish in Guinea-Bissau. *Aquat. Conserv. Mar. Freshw. Ecosyst.* **25**, 411–430.

Lehman, R. N., Poulakis, G. R., Scharer, R. M., Hendon, J. M., Court, A. G., Wooley, A. K., Williams, A. M., Ajemian, M. J., Hadden, J. P., Beal, J. L., McCallister, M. P., & Phillips, N. M. (2022). Environmental DNA evidence of the Critically Endangered smalltooth sawfish, *Pristis pectinata*, in historically occupied US waters. *Aquat. Conserv. Mar. Freshw. Ecosyst.* **32**, 42–54.

Lehman, R. N., Poulakis, G. R., Scharer, R. M., Schweiss, K. E., Hendon, J. M., & Phillips, N. M. (2020). An environmental DNA tool for monitoring the status of the Critically Endangered Smalltooth Sawfish, *Pristis pectinata*, in the western Atlantic. *Conserv. Genet. Resour.* **12**, 621–629.

Longo, F. (1882). Il canale di Messina e le sue correnti: con appendice sui pesci che lo popolano. Tipografia Ribera, Messina.

Lozano, R. (1928). Ictiologia Iberica (Fauna Iberica). Peces (Generalidades, Ciclostomos y Elasmobranquitos). Mus. Nac. Cienc. Nat. Madrid, 1, 692.

McClenachan, L., Cooper, A. B., & Dulvy, N. K. (2016). Rethinking Trade-Driven Extinction Risk in Marine and Terrestrial Megafauna. *Curr. Biol.* **26**, 1640–1646.

Miller, W. A. (1974). Observations on the Developing Rostrum and Rostral Teeth of Sawfish: Pristis perotteti and P. cuspidatus. *Copeia* **1974**, 311.

Morgan, D. L., Allen, M. G., Ebner, B. C., Whitty, J. M., & Beatty, S. J. (2015). Discovery of a pupping site and nursery for critically endangered green sawfish *Pristis zijsron*. *J. Fish Biol.* **86**, 1658–1663.

Morgan, D. L., Whitty, J. M., Phillips, N. M., Thorburn, D. C., Chaplin, J. A., & McAuley, R. (2011). North-western Australia as a hotspot for endangered Elasmobranchs with particular reference to sawfishes and the Northern river Shark. *J. R. Soc. West. Aust.* **94**, 345–358.

Morgan, D. L., Ebner, B. C., Allen, M. G., Gleiss, A. C., Beatty, S. J., & Whitty, J. M. (2017). Habitat use and site fidelity of neonate and juvenile green sawfish *Pristis zijsron* in a nursery area in Western Australia. *Endanger. Species Res.* **34**, 235–249.

Morgan, D. L., Lear, K. O., Dobinson, E., Gleiss, A. C., Fazeldean, T., Pillans, R. D., Beatty, S. J., & Whitty, J. M. (2021). Seasonal use of a macrotidal estuary by the endangered dwarf sawfish, *Pristis clavata*. *Aquat. Conserv. Mar. Freshw. Ecosyst.* **31**, 2164–2177.

Morgan, D. L., Wueringer, B. E., Allen, M. G., Ebner, B. C., Whitty, J. M., Gleiss, A. C., & Beatty, S. J. (2016). What Is the Fate of Amputee Sawfish? *Fisheries* **41**, 71–73.

Norton, S. L., Wiley, T. R., Carlson, J. K., Frick, A. L., Poulakis, G. R., & Simpfendorfer, C. A. (2012). Designating Critical Habitat for Juvenile Endangered Smalltooth Sawfish in the United States. *Mar. Coast. Fish.* **4**, 473–480.

Peverell, S. C. (2005). Distribution of sawfishes (Pristidae) in the Queensland Gulf of Carpentaria, Australia, with notes on sawfish ecology. *Environ. Biol. Fishes* **73**, 391–402.

Phillips, N. M., Chaplin, J. A., Peverell, S. C., & Morgan, D. L. (2016). Contrasting population structures of three Pristis sawfishes with different patterns of habitat use. *Mar. Freshw. Res.* **68**, 452–460.

Phillips, Nicole M., Fearing, A., & Morgan, D. L. (2017). Genetic bottlenecks in Pristis sawfishes in northern Australian waters. *Endanger. Species Res.* **32**, 363–372.

Poulakis, G. R., & Grubbs, R. D. (2019). Biology and ecology of sawfishes: global status of research and future outlook. *Endanger. Species Res.* **39**, 77–90.

Poulakis, G. R., & Seitz, J. C. (2004). Recent occurrence of the smallthooth sawfish, *Pristis pectinata* (Elasmobranchiomorphii: Pristidae) in Florida Bay and the Florida Keys, with comments on sawfish ecology. *Fla. Sci.* **67**, 27–35.

Poulakis, G. R., Stevens, P. W., Timmers, A. A., Stafford, C. J., & Simpfendorfer, C. A. (2013). Movements of juvenile endangered smalltooth sawfish, *Pristis pectinata*, in an estuarine river system: use of non-main-stem river habitats and lagged responses to freshwater inflow-related changes. *Environ. Biol. Fishes* **96**, 763–778.

Prohaska, B. K., Bethea, D. M., Poulakis, G. R., Scharer, R. M., Knotek, R., Carlson, J. K., & Grubbs, R. D. (2018). Physiological stress in the smalltooth sawfish: effects of ontogeny, capture method, and habitat quality. *Endanger. Species Res.* **36**, 121–135.

Rafinesque, C. (1810). Indice d'ittiologia Siciliana. Messina.

Reis-Filho, J. A., Freitas, R. H. A., Loiola, M., Leite, L., Soeiro, G., Oliveira, H. H. Q., Sampaio, C. L. S., Nunes, J. de A. C. C., & Leduc, A. O. H. C. (2016). Traditional fisher perceptions on the regional disappearance of the largetooth sawfish Pristis pristis from the central coast of Brazil. *Endanger. Species Res.* **29**, 189–200.

Romero, A. (2012). New Approaches to the Study of Marine Mammals, chap. When Whales Became Mammals: The Scientific Journey of Cetaceans From Fish to Mammals in the History of Science. Agricultural and Biological Sciences

Saitta, E. (1902). Pesci e molluschi dei mari della Sicilia, con aggiunte dei piu comuni crostacei ed altri animali d'acqua salsa: vocabolario siciliano-italiano e italiano-siciliano. Tip. Del Progresso, Messina.

Sani, L. M. I., Husna, A. K., Subhan, B., & Madduppa, H. (2021). Environmental DNA (eDNA) reveals endangered narrow sawfish across Indonesian Reefs. *IOP Conf. Ser. Earth Environ. Sci.* **944**, 012020.

Seitz, J. C., & Hoover, J. J. (2017). Taxonomic resolution of sawfish rostra from two private collections. *Endanger. Species Res.* **32**, 525–532.

Simpfendorfer, C. (2013). *Pristis zijsron* (errata version published in 2019) *IUCN Red List of Threatened Species.*

Simpfendorfer, C. A. (2000). Predicting Population Recovery Rates for Endangered Western Atlantic Sawfishes Using Demographic Analysis. *Environ. Biol. Fishes* **58**, 371–377.

Simpfendorfer, C. A., & Kyne, P. M. (2009). Limited potential to recover from overfishing raises concerns for deep-sea sharks, rays and chimaeras. *Environ. Conserv.* **36**, 97–103.

Simpfendorfer, C. A., Kyne, P. M., Noble, T. H., Goldsbury, J., Basiita, R. K., Lindsay, R., Shields, A., Perry, C., & Jerry, D. R. (2016). Environmental DNA detects Critically Endangered largetooth sawfish in the wild. *Endanger. Species Res.* **30**, 109–116.

Thorburn, D. C., Morgan, D. L., Rowland, A. J., Gill, H. S., & Paling, E. (2008). Life history notes of the critically endangered dwarf sawfish, *Pristis clavata*, Garman 1906 from the Kimberley region of Western Australia. *Environ. Biol. Fishes* **83**, 139–145.

Tortonese, E. (1956). Leptocardia, Ciclostomata, Selachii. vol. 2 of Fauna d'Italia. Edizioni Calderini, Bologna.

White, T. (2002). The Book of Beasts: Being a Translation from a Latin Bestiary of the Twelfth Century. University of Wisconsin digital collections. Parallel Press.

White, W. T., & Kyne, P. M. (2010). The status of chondrichthyan conservation in the Indo-Australasian region. *J. Fish Biol.* **76**, 2090–2117.

White, W. T., Appleyard, S. A., Kyne, P. M., & Mana, R. R. (2017). Sawfishes in Papua New Guinea: a preliminary investigation into their status and level of exploitation. *Endanger. Species Res.* **32**, 277–291.

Whitehead, P., Bauchot, M., Hureau, J., Nielsen, J. & Tortonese, E. (1984). Fishes of the North-eastern Atlantic and the Mediterranean, Vol. 1. Unaited Nation Educational Scientific and Cultural Association, 7 Place de Fontenoy, 75700 Paris.

Whitty, J. M., Keleher, J., Ebner, B. C., Gleiss, A. C., Simpfendorfer, C. A., & Morgan, D. L. (2017). Habitat use of a Critically Endangered elasmobranch, the largetooth sawfish Pristis pristis, in an intermittently flowing riverine nursery. *Endanger. Species Res.* **34**, 211–227.

Whitty, J. M., Morgan, D. L., Peverell, S. C., Thorburn, D. C., Beatty, S. J., Whitty, J. M., Morgan, D. L., Peverell, S. C., Thorburn, D. C., & Beatty, S. J. (2009). Ontogenetic depth partitioning by juvenile freshwater sawfish (Pristis microdon: Pristidae) in a riverine environment. *Mar. Freshw. Res.* **60**, 306–316.

Whitty, J. M., Phillips, N. M., Thorburn, D. C., Simpfendorfer, C. A., Field, I., Peverell, S. C., & Morgan, D. L. (2014). Utility of rostra in the identification of Australian sawfishes (Chondrichthyes: Pristidae). *Aquat. Conserv. Mar. Freshw. Ecosyst.* **24**, 791–804.

Wueringer, B. E. (2012). Electroreception in Elasmobranchs: Sawfish as a Case Study. *Brain. Behav. Evol.* **80**, 97–107.

Wueringer, B. E., Squire, L., & Collin, S. P. (2009). The biology of extinct and extant sawfish (Batoidea: Sclerorhynchidae and Pristidae). *Rev. Fish Biol. Fish.* **19**, 445–464.

Yan, H. F., Kyne, P. M., Jabado, R. W., Leeney, R. H., Davidson, L. N. K., Derrick, D. H., Finucci, B., Freckleton, R. P., Fordham, S. V., & Dulvy, N. K. (2021). Overfishing and habitat loss drive range contraction of iconic marine fishes to near extinction. *Sci. Adv.* **7**, eabb6026.

Chapter 2

Integrated molecular and morphometric taxonomy and species diversity of historical Mediterranean sawfish rostra

2.1 Introduction

Sharks, rays, and chimaeras are nowadays extremely threatened by human impacts, such as fisheries exploitation and habitat degradation (Dulvy et al., 2021). Declines in population abundance and reduction of range brought many species to the brink of extinction (Dulvy et al., 2014; Simpfendorfer & Dulvy, 2017). Sawfishes (family Pristidae) are among the most affected elasmobranch groups by these impacts. In the last decade, growing attention is focused on sawfish's conservation to preserve the remaining few populations in the world, thus limiting their extinction. There are five extant sawfish species across the globe: *Anoxypristis cuspidata* (Latham, 1794), *Pristis pristis* (Linnaeus, 1758), *Pristis zijsron* (Bleeker, 1851), *Pristis pectinata* (Latham, 1794), and *Pristis clavata* (Garman, 1906). These have all drastically declined in abundance in many coastal areas worldwide, mainly due to overfishing and habitat loss (Dulvy et al., 2016; Yan et al., 2021). Because of their life-history traits, which determine a low reproductive potential, they are particularly susceptible and cannot sustain even a minimal external pressure, such as bycatch and habitat loss.

In the Mediterranean Sea, the reconstruction of the historical abundance and distribution of such extremely vulnerable species have been a challenge due to the long history of heavy human exploitation (Ferretti et al., 2016). With the help of museum collections and bibliographical records analysis, Ferretti et al. (2016) reconstructed the historical recording of sawfish in the Mediterranean

Sea, providing evidence that two species of sawfish, *P. pristis* and *P. pectinata,* occurred in the basin and went extinct before the 1970s. Although in the Mediterranean the water temperature can reach winter minima below the thermal tolerance for the species (Poulakis et al., 2011; 2013; Simpfendorfer et al., 2011), recent investigations revealed that habitat requirements for these species might be broader than previously known (i.e., association with grass beds, Moore et al., 2014) suggesting that there might be other aspects of sawfish historical ecology that still need to be discovered.

Hence, the current rarity of sawfishes and their conservation status make specimens conserved in museum collections (e.g., dried rostra) extremely important for recovering data to reconstruct their historical biogeography and ecology (Seitz & Hoover, 2017). However, these precious records typically lack of reliable data about the catch of the original individual (e.g., locality, date) since the extensive trade of rostra among public and private collectors in the past. In addition, the correct species identification using only the rostrum is challenging for juveniles (Whitty et al., 2014). To avoid the risk of misidentification and to promote sawfish conservation, each organism must be associated to the correct taxonomy (Mace, 2004). Analyses of rostrum morphometrics have been proven an efficient method to discriminate sawfish species (Faria et al., 2013; Whitty et al., 2014; Seitz & Hoover, 2017; Trif & Vonica, 2018). Geometric morphometrics based on landmarks also has represented a valid instrument for discriminating species (Adams, Rohlf, & Slice, 2004). The combination of both morphological approaches with molecular taxonomy can considerably improve elasmobranch species identification (Moftah et al., 2011; Petean, Naylor, & Lima, 2020; Bellodi et al., 2022).

However, tissues not specifically stored for genetic analyses, such as the cartilage of sawfish museum specimens, can yield lower quantities and more degraded DNA than properly preserved ones (Wandeler, Hoeck, & Keller, 2007). Ancient DNA (aDNA) technologies are nowadays considered adequate tools to understand the history of rare and endangered species. One of the main issues of aDNA analysis is the DNA damage of old and dried museum finds, such as degradation and fragmentation (Pääbo, 1989). In addition, usually, only a small proportion of surviving copies of endogenous DNA is available in non-modern samples, thus allowing the contamination from exogenous DNA molecules present in the surface of the sample or introduced during laboratory processes (see Puncher et al., 2019). Despite all these challenges in aDNA extraction and amplification, molecular approaches have helped to discriminate between sawfish species, demonstrating that tissues from dry rostra were a reliable source of DNA (Phillips et al., 2009; Faria et al., 2013).

This chapter deals with the taxonomic inventory of sawfish rostra specimens present in the natural history museums of the Mediterranean area started by Ferretti et al. (2016). Specifically, the objectives were to verify the sources of bibliographic accounts, to correctly identify at the species level the rostra, to uncover more information regarding the capture of the specimen mentioned in the literature and improve our knowledge about the distribution and ecology of past sawfish populations.

2.2 Materials and Methods

2.2.1 Searching and sampling of museum rostra

A thorough literature search was carried out to find sawfish rostra in natural history museums or private collections across the Mediterranean area. Starting from the capillary bibliographic search and the investigation on museum collections conducted by Ferretti et al. (2016), all available collections were accurately investigated by contacting curators to find out other Mediterranean structures that possessed sawfish rostra finds.

A total of 23 European museums and four private collections returned positive feedback giving suitable pictures, morphometric measures, or powder tissue specimens of 172 sawfish conserved in their collections (Table 1). Sampling was accompanied by further research conducted to find as much information as possible on sawfish remains, such as the geographical origin and year of sawfish capture.

For molecular analysis, we identified the ventral surface at the base of the rostrum as the most suitable part to sample cartilage, to avoid affecting the expositional value of the museum exhibit. Before cartilage sampling, on-site, rostra were superficially decontaminated with 1.5% sodium hypochlorite and with a slight abrasion with sandpaper and the superficial layers was removed. Then, the sampling surface was exposed to UV radiation for a minimum of 15 minutes by a portable UV light lamp. Small holes were produced with a drill at the base of each rostrum to gain access to the internal matrix and extract cartilage powder. All the instruments used were cleaned after each sample with bleach and ethanol. We collected the cartilage powder of each sample into a sterile screw tube and stored it until laboratory procedures.

Table 1 List of museum and private collections which contributed with samples of rostra and detail of total rostra number involved in each analysis.

			Morphology		Molecular analysis	
Museums and private collections	**City (Country)**	**N**	**Landmarks**	**Measures**	**NADH2**	**COI**
Natural History Museum of Trieste	Trieste (Italy)	49	48	49	44	44
Natural History Museum of Venezia	Venezia (Italy)	27	25	27	-	-
Museum of Natural History "La Specola", University of Firenze	Firenze (Italy)	20	17	20	19	19
Wilderness s.n.c Studi Ambientali	Palermo (Italy)	15	15	15	15	15
Natural History Museum of Voghera	Voghera (Italy)	7	6	6	7	7
Zoological Museum "P. Doderlein", University of Palermo	Palermo (Italy)	5	3	5	3	3
Natural History Museum of Dubrovnik	Dubrovnik (Croatia)	4	4	4	4	4
Natural History Museum of Rovereto	Rovereto (TN, Italy)	4	4	4	4	4
Natural History Museum, University of Pisa	Pisa (Italy)	4	4	4	4	4
Zoological Collection, University of Bologna	Bologna (Italy)	4	4	4	1	1
Museum of Comparative Anatomy "Battista Grassi", Sapienza	Roma (Italy)	3	3	3	3	3
Museum of Zoology, University of Navarra	Navarra (Spain)	3	3	3	3	3
Natural History Museum of Verona	Verona (Italy)	3	3	3	3	3
Comparative anatomy collection, University of Bologna	Bologna (Italy)	2	-	-	2	2
Croatian Museum of Natural History	Zagreb (Croatia)	2	-	2	-	-
Museo Natura	Sant'Alberto	2	2	2	-	-
Museum of Zoology, University of Padova	Padova (Italy)	2	2	2	2	2
National Museum of Zadar, Department of Natural History	Zadar (Croatia)	2	1	2	1	1
Natural History Museum of Rijeka	Rijeka (Croatia)	2	2	2	2	-
Natural History Museum of Split	Split (Croatia)	2	2	2	2	1
Regional Museum of Terrasini	Palermo (Italy)	2	2	2	2	2
Casa Matha	Ravenna (Italy)	1	1	1	1	1
Natural History Museum of Comiso	Comiso (RG, Italy)	1	1	1	-	-
Private collection (CP)	Fano (PU, Italy)	3	3	3	3	3
Private collection (IM)	Zadar (Croatia)	1	1	1	-	-
Private collection (J)	Venezia (Italy)	1	-	1	-	-
Private collection (PB)	Firenze (Italy)	1	1	1	1	1
		172	**157**	**169**	**126**	**123**

2.2.2 Molecular analyses

2.2.2.1 Laboratory procedures and DNA extraction

All laboratory procedures followed high sterility standards and appropriate criteria to prevent contamination by exogenous DNA (Cooper & Poinar, 2000; Gilbert et al., 2005; Llamas et al., 2017). DNA extraction and pre-PCR set up of the samples were performed in physically separated and designated areas (pre-PCR lab) at the Laboratory of Ancient DNA of the Department of Cultural Heritage (University of Bologna, Ravenna Campus) exclusively reserved for ancient DNA analysis (Fulton, 2012). All the surfaces of non-disposable equipment and instruments were cleaned using bleach and ethanol or by DNA-Exitus Plus™ cleaning kit (Applichem Inc., Omaha, NE, USA). All the reagents used during the DNA extraction or PCR set-up, as well as all the plastic labware, were exposed to UV radiation for 30 minutes before their use (except for DNA polymerase, primers, and dNTPs). Suitable disposable clothing (full body suit, hair cap, boots, face mask, face shield, arm covers and two pairs of gloves) were worn during the analyses of ancient samples in the pre-PCR facility.

Total genomic DNA (henceforth gDNA) was isolated starting from 100-150 mg of powder tissue using the MinElute PCR Purification Kit (Qiagen) following the chemical extraction protocol (Serventi et al., 2018) and accurately improved starting from previously published studies (Dabney, Meyer, & Pääbo, 2013; Allentoft et al., 2015). Total gDNA was eluted in 50 µL of TET buffer (10 mMTris-HCl, 1 mM EDTA, 0.05% Tween-20). Then, 25% of samples were extracted and amplified twice to verify the authenticity of the results. To avoid contaminations by amplicons, PCR amplifications were conducted in a physically separated facility, dedicated to post-PCR procedures.

2.2.2.2 DNA amplification and sequencing

Due to the highly fragmented nature of aDNA, two overlapping amplicons (of about 150 bp each) were identified from the nicotinamide dehydrogenase subunit 2 (NADH2) and two from the Cytochrome Oxidase subunit 1 (COI). These short mitochondrial regions have been thought to be consecutive in each mitochondrial marker to allow the combination into longer fragments. These regions were targeted by building two reference datasets: we retrieved all available NADH2 (N = 17) and COI (N = 79) sequences belonging to the five target species from both the BOLD Systems (https://www.boldsystems.org) and the NCBI (http://www.ncbi.nlm.nih.gov/) online repositories (Table S1). In addition, 92 mitogenomes of *P. pristis* by Feutry et al. (2015) were used as reference and added to the datasets of the corresponding marker. The identified regions of 350 bp (NADH2)

and 320 bp (COI), containing 72 and 86 variable sites, respectively, were selected for subsequent analyses.

New primer pairs were developed using Primer3 (Untergrasser et al., 2012). Careful consideration was given to the physical and structural properties of the oligonucleotides, such as annealing temperature, guanine and cytosine content, and tendency towards self-complementary binding. All primer pairs were then evaluated using the PCR simulation software Amplifix v.1.7.0 (http://crn2m.univ-mrs.fr/pub/amplifx-dist). Primer codes, sequences, primer pair annealing temperatures, and length of the target sequence are listed in Table S2.

PCR reactions were performed in 25 µL reactions, containing 4 µL of gDNA, 1X PCR Gold Buffer (ThermoFisher®), 1.5 mM of $MgCl_2$ (ThermoFisher®), 0.25 mM of dNTP mix, 0.8 mg/µL of BSA, 0.2 µM of each primer and 0.25 U of AmpliTaq Gold DNA polymerase (ThermoFisher®). Amplifications were performed using PCR 2720 Thermocyclers. The following conditions were used: an initial DNA denaturation at 95°C for 10 min followed by 50 cycles at 95°C for 15 sec, 50°-55°C for 30 sec, 72°C for 1 min, followed by a final extension of 5 min at 72°C. Amplification products were checked on a 1.5% agarose gel. Amplicons were enzymatically purified using the ExoSAP Express PCR Product Cleanup Reagent following the manufacturer's instructions (https://www.thermofisher.com/order/catalog/product/75001.200.UL). Sequencing was performed by a commercial sequence service provider (Macrogen Europe B.V., Amsterdam, the Netherlands) employing the same primers used for the PCR. The samples were sequenced in both directions.

2.2.2.3 Species delimitation and specimen identification

Trace files were manually checked and edited with the software MEGA v.7.0 (Kumar, Stecher, & Tamura, 2016). The newly obtained sequences were added to the reference NADH2 and COI datasets and were aligned using the ClustalW algorithm (Thompson, Higgins, & Gibson, 1994) implemented in MEGA v.7.0. Both final datasets were analysed using different tree-based approaches: Neighbour-Joining (NJ), Maximum Likelihood (ML) and Bayesian inference (BI). JModelTest v.2.1 software (Darriba et al., 2012) was used to select the most appropriate evolutionary models based on the Bayesian information criterion (BIC). The resulting TrN+I and HKY+G models for NADH2 and COI datasets, respectively, were used to perform the subsequent analyses. The NJ tree clustering (Saitou & Nei, 1987) was obtained with MEGA v.7.0 (Kumar, Stecher, & Tamura, 2016) with bootstrap support of 1,000 replicates (Felsenstein, 1985). PhyML v.3.0 online (http://www.atgc-montpellier.fr/phyml; Guindon et al., 2010) was used to carry out the ML analysis with the bootstrap confidence determined by performing 1000 replicates. To perform the BI, MrBayes v.3.2.7 (Ronquist et al., 2012) was used with an MCMC analysis conducted for two runs in parallel with random starting

trees, for 500,000 generations and sampled every 5000. The chain was estimated by stable split-standard deviations between the two runs and stable sampled log-likelihood values. The burn-in was set to the first 25% of generations. The homologous sequence of *Leucoraja erinacea* (Rajidae; GenBank code JQ034406) was added to the final datasets as an outgroup. Tree editing with bootstrap and posterior probability values was done in TreeGraph v.2 (Stöver & Müller, 2010).

Results of the tree-based approaches were further compared with two species delimitation methods to infer the number of groups (species) in both datasets and consequently to attribute each new sequence at the correct species (Collins & Cruickshank, 2013). The first distance-based method was the Assemble Species by Automatic Partitioning (ASAP; Puillandre, Brouillet, & Achaz, 2021) carried out using the web interface (https://bioinfo.mnhn.fr/abi/public/asap/) with default maximum intraspecific distance. The second method applied was the Bayesian implementation of the Poisson tree processes (bPTP; Zhang et al., 2013) conducted on the webserver (http://species.h-its.org/ptp) using 100,000 MCMC generations, a thinning interval of 100% and 10% of burn-in. The Bayesian trees were used as input for the bPTP analysis.

2.2.3 Morphometric analysis

2.2.3.1 Traditional approach based on linear measurements

Linear measurements (Figure 1) were taken following protocols of Faria et al. (2013), Whitty et al. (2014), Seitz & Hoover (2017) and Trif & Vonica (2018) and consisted of: (1) total specimen length (TSL), (2) total rostrum length (TRL), (3) standard rostrum length (SRL), (4) proximal rostrum width (PRW), equivalent to SRW in Whitty et al. (2014); (5) distal rostrum width (DRW); (6) proximal rostral tooth gap (PTG); (7) distal rostral tooth gap (DTG). A total of 34 specimens without the rostrum−head juncture were treated following Seitz & Hoover (2017) and TRL was assumed to be equivalent to TSL for this study. Following the same approach of Seitz & Hoover (2017), values of morphometric variables were standardized as ratios and were expressed in relation to TRL by dividing each measurement to TRL and multiplying by 100. Rostral teeth were also counted and, when missing, the tooth counts were based on the presence of empty alveoli.

To confirm the assignment to a given species, a larger dataset was created compiling available public data on the same linear measures of sawfish rostra from the East Atlantic (Robillard & Séret, 2006), from private collections (Seitz & Hoover, 2017) and the Natural History Museum of Sibiu (Trif & Vonica, 2018). This larger dataset was used to compute the linear discriminant analysis (LDA) with the function *lda* and the MASS package (Venables & Ripley, 2002) on R version 4.1.0 (R Core

Team, 2021). The LDA was based on five standardized measures (TRL, SRL, PRW, DRW, PTG and DTG) using the molecular identification as a prior assignment to species. The model finds the directions (linear discriminants) that maximize the separation between groups (species); then these linear combinations of predictor variables were used to classify each rostrum to the predicted species with posterior probabilities.

Descriptive statistics (i.e., range, mean and standard error) of standardized linear measurements were calculated for each resulting species to determine ranges of these variables useful to compare with other sawfish populations. Following the relation between rostrum length and total animal length (Morgan et al., 2011; Faria et al., 2013), we estimated the maturity state of each sample.

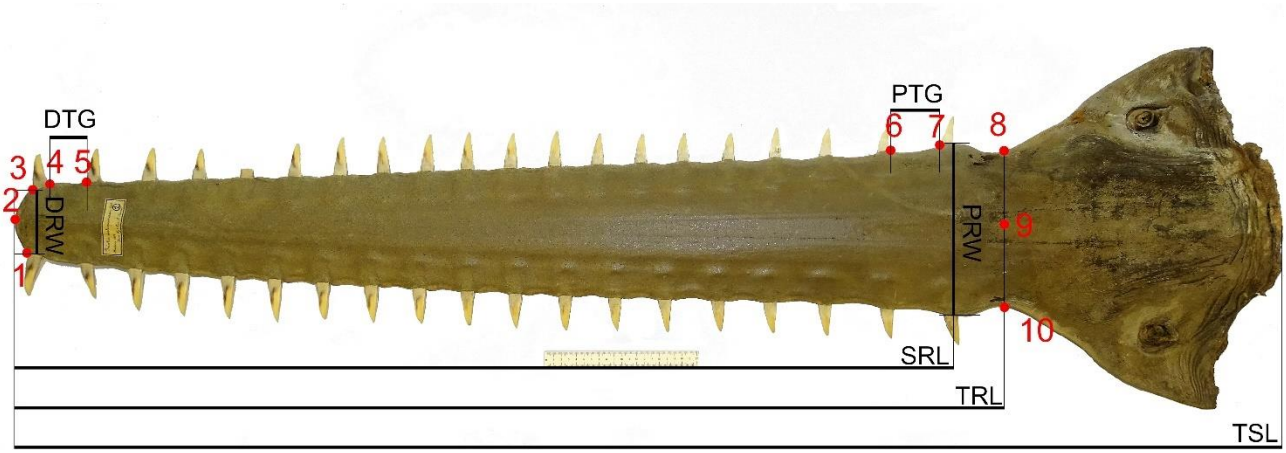

Figure 1 Linear measurements and Landmarks utilised for the morphometric analyses of rostra.
TSL, total specimen length; TRL, total rostrum length; SRL, standard rostrum length; PRW, proximal rostrum width; DRW, distal rostrum width; PTG, proximal rostral tooth gap; DTG, distal rostral tooth gap.
Landmarks are placed as follow: (1) at the base of the most distal rostral tooth (left side of the rostrum); (2) at the rostrum tip; (3) and (4) at both base of the most distal rostral tooth (right side of the rostrum); (5) at the base of the second distal rostral tooth (right side of the rostrum); (6) at the base of the second most proximal rostral tooth (right side of the rostrum); (7) at the base of the first most proximal rostral tooth (right side of the rostrum); (8) at the right side where the rostrum begins to flare; (9) at mid-point where the rostrum begins to flare; (10) at the left side where the rostrum begins to flare.

2.2.3.2 Geometric approach based on landmarks

Available images of rostra were properly checked to verify their suitability for the landmark-based morphometric methods. In total, 157 images were finally selected for the analysis. Ten landmarks (Figure 1) were selected *a priori* considering the most variable section of the rostrum among sawfish species (Faria et al., 2013; Whitty et al., 2014; Seitz & Hoover, 2017). Each picture was rotated to have the same direction of the rostrum tip. For each image, the measure was set using the scale on the metric reference contained in the picture. When the metric reference was missing, the distance from the two most distal teeth was used as a reference.

Landmarks were placed as reported in the legend of Figure 1. Since in 32% of specimens the rostrum−head juncture was not apparent, the last three landmark points were taken at the beginning of the specimen, assumed to be equivalent to the beginning of the rostrum. A TPS file was created to store all the photo samples, using the free software tpsUtil (Rohlf, 2015).

Landmarks were digitalized using tpsDig2 v2.31 (Rohlf, 2006). The free software MorphoJ v1.07 (Klingenberg, 2011) was used to perform Procrustes analysis, integrating as classifiers genus and species and as covariates PTG and DTG. With the Procrustes analysis, all pictures were translated, rotated, and scaled to obtain the final superimposed image, which allowed the comparison of landmarks. To check the landmark's repeatability a scattering plot was obtained. Subsequently, the covariance matrix was created to perform the Principal Component Analysis (PCA) that allowed to graphically visualize the shape variation of the dataset. Using a Procrustes ANOVA (analysis of variance: Klingenberg & McIntyre, 1998; Klingenberg, Barluenga, & Meyer, 2002) the variance was partitioned in size or shape among individuals (i.e., averaged replicas representing the 'true' sample variance) and residual component (i.e., variation among replicas).

2.3 Results

A total of 172 museum specimens were retrieved from the museum and private collections (Table 1). Unfortunately, the search for associated information has been characterized by a general lack of data. Most of the samples were recorded without information on the geographical provenance (70%) and on the year of capture (79%). When present, the documented geographical origin was registered as Indian Ocean (N=19), Mediterranean (N=15), Atlantic (N=3) and West Pacific (N=2), while the temporal range of catches was registered between 1600 and 1959. The complete list of the samples with associated information is provided in Table S3. The 16 samples tagged with indication of Mediterranean origin were identified in this study as follows: seven individuals have been identified as *P. zijsron*, five as *A. cuspidata*, three as *P. pectinata* and one as *P. pristis* (Table 2). Of these, 12 were part of the 21 museum specimens previously inventoried by Ferretti et al. (2016) in their list of museum exhibits (see Table 1 of Ferretti et al., 2016).

Table 2 – List of the 16 specimens labelled as Mediterranean with details on their origin, year and identification. Specimens inventoried by Ferretti et al. (2016) are indicated.

Inventory label	Museum	Documented geographical origin	Year	Museum identification	This study identification	Inventoried by Ferretti et al. (2016)
100944	Comparative anatomy collection, University of Bologna	Mediterranean Sea, Adriatic	-	*P. marsilii*	*P. zijsron*	No
6112	Museum of Natural History "La Specola", University of Firenze	Mediterranean Sea, Messina	1837	*P. pectinata*	*P. pectinata*	Yes (number 4)
107 129	Museum of Zoology, University of Navarra	Mediterranean Sea	-	*P. pristis*	*A. cuspidata*	Yes (number 14)
107 127	Museum of Zoology, University of Navarra	Mediterranean Sea	-	*P. pectinata*	*P. zijsron*	Yes (number 17)
107 145	Museum of Zoology, University of Navarra	Mediterranean Sea	-	*P. pristis*	*P. zijsron*	Yes (number 16)
P29e	Museum of Zoology, University of Padova	Mediterranean Sea, Adriatic	<1730	*Pristis spp.*	*P. pectinata*	No
P28e	Museum of Zoology, University of Padova	Mediterranean Sea, Adriatic	<1730	*Pristis spp.*	*P. zijsron*	No
PMD 20	Natural history museum of Dubrovnik	Mediterranean Sea, Gruž, Dubrovnik	-	*P. pectinata*	*P. zijsron*	Yes (number 12)
PES 00018	Natural history museum of Rovereto	Mediterranean Sea, Venezia market	1900	*P. pectinata*	*A. cuspidata*	Yes (number 1)
PES 00019	Natural history museum of Rovereto	Mediterranean Sea, Genova market	1900	*P. pectinata*	*A. cuspidata*	Yes (number 2)
PES 00020	Natural history museum of Rovereto	Mediterranean Sea, Genova market	1900	*Pristis spp.*	*P. zijsron*	Yes (number 3)
PMST 10	Natural history museum of Split	Mediterranean Sea, Southern Adriatic	1901	*P. pectinata*	*P. zijsron*	Yes (number 20)
Pe 0116	Natural History Museum, University of Pisa	Mediterranean Sea	-	*P. pectinata*	*A. cuspidata*	Yes (number 5)
-	Regional Museum of Terrasini	Mediterranean Sea	-	*P. pristis*	*P. pristis*	No
AN184	Zoological Museum "P. Doderlein", University of Palermo	Mediterranean Sea, Palermo	<1895	*Pristis spp.*	*P. pectinata*	No
AN182	Zoological Museum "P. Doderlein", University of Palermo	Mediterranean Sea, Palermo	-	*Pristis spp.*	*A. cuspidata*	No

2.3.1 Species delimitation and specimen identification based on molecular tools

Total aDNA was successfully extracted from 129 dried rostra and no contamination was observed in any extractions or PCR negative controls, supporting the quality of the data obtained. Overall, 126 sequences of NADH2 and 123 of COI gene fragments were successfully obtained. The remaining sequences (three NADH2 and six COI) were excluded due to the low quality of trace files likely caused by the extremely degraded tissue. Data were also validated by the congruence among replicates of extractions and amplifications. Final sequence alignments were 350 bp and 320 bp in total length for NADH2 and COI datasets, respectively.

Phylogenetic trees based on NADH2 and COI sequences showed well-defined species-specific clusters with high support of bootstrap and posterior probabilities in which all new sequences were bundled with references (Figure 2). Bootstrap values of each species-specific cluster were equal to 99% in the NJ tree of NADH2 while ranging from 95% (*P. zijsron*) to 100% in the NJ tree of the COI fragment. The posterior probabilities of the BI analysis ranged from 0.9 (*P. zijsron*) to 1.0 using the NADH2 dataset and were equal to 1.0 in each cluster using the COI dataset. Relatively lower values of bootstrap resulted in the ML analysis, ranging from 73% (*P. zijsron*) to 100% (*P. clavata*) for NADH2 and from 73% (*P. zijsron*) to 99% (*P. pectinata*) in the COI marker.

Accordingly, the ASAP species delimitation analysis obtained the best asap-score (1.0 and 2.5 respectively for NADH2 and COI) when grouping sequences in five species-specific clusters (Figure 2). In contrast, the bPTP delimitation analysis found seven different groups in both datasets, assigning two groups within the species *A. cuspidata* and *P. pristis*, while the other groups were species-specific (Figure 2). According to molecular results, 30 rostra were identified as *A. cuspidata*, 16 as *P. pectinata*, 19 as *P. pristis* and 61 as *P. zijsron.* Overall, 68 out of 172 rostra had not previously been identified at the species level and 34 rostra had been misidentified, mainly involving the species *P. zijsron*, previously erroneously labelled as *P. pectinata.*

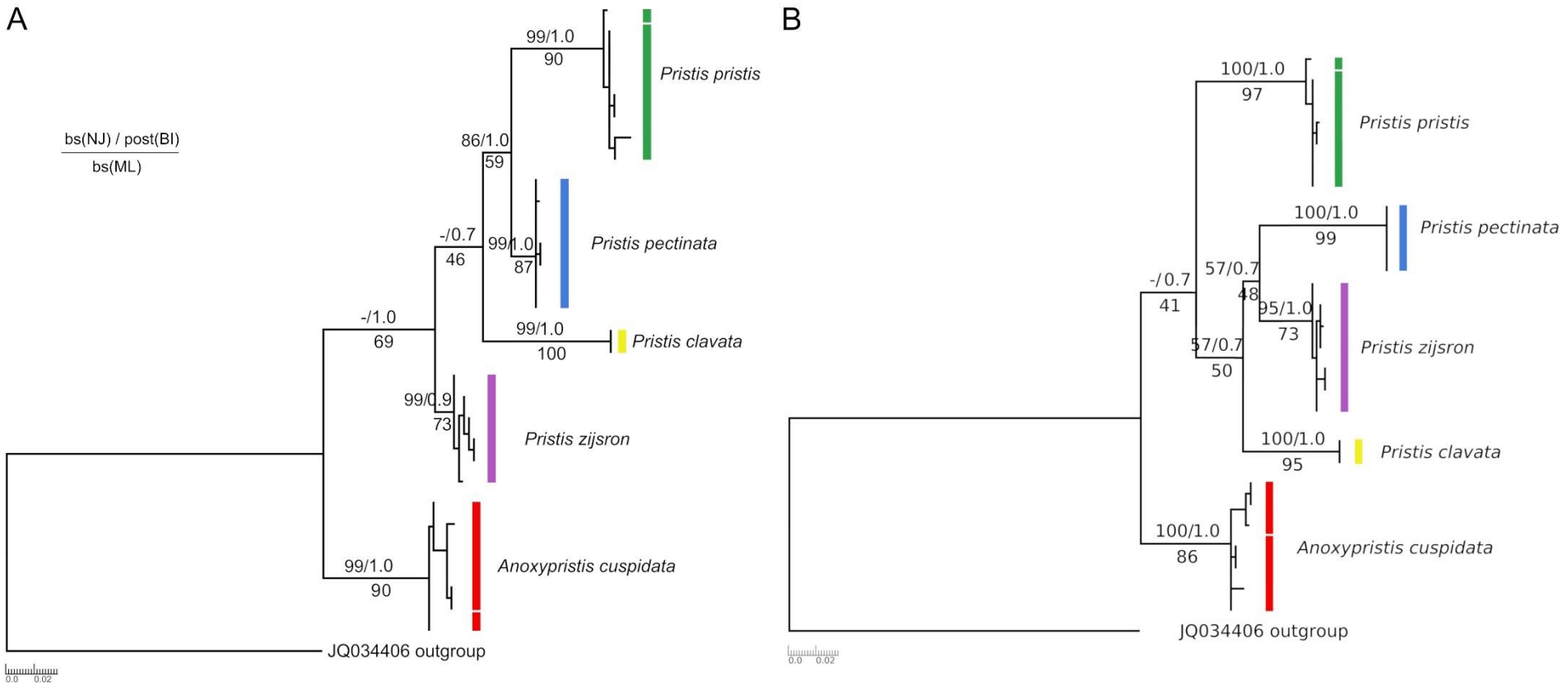

Figure 2 Results of the neighbour-joining (NJ), Bayesian inference (BI) and maximum-likelihood (ML) trees of sawfish species inferred from the mitochondrial NADH2 (A) and COI (B) datasets. Support values for each node are represented by Bayesian posterior probabilities (post), NJ and ML bootstrap values (bs). Each vertical bars represents bPTP analysis results (seven groups) while different colours are in accord with the ASAP results (five groups).

2.3.2 Morphometric analyses

Linear measurements were taken on 169 rostra out of 172 (Table 1) since three specimens were too damaged and with missing parts.

Using the larger dataset created including all available data from previous studies, the first discriminant axis of the LDA explained 85.43% of the variation among groups and the second 13.56% (Figure 3). The overall classification accuracy of LDA was 97.08%, with higher scores obtained for *A. cuspidata* and *P. pristis* with that achieved the highest accuracy (100%) compared with *P. pectinata* and *P. zijsron* that achieved the lowest accuracy (95.56% and 94.51% respectively) (Table 4). Among the new rostra analysed, four belonged to juveniles of *P. zijsron* and were morphologically classified as the 'wrong' species *P. pectinata*, which corresponds to an error rate of 5.41% (Table 3).

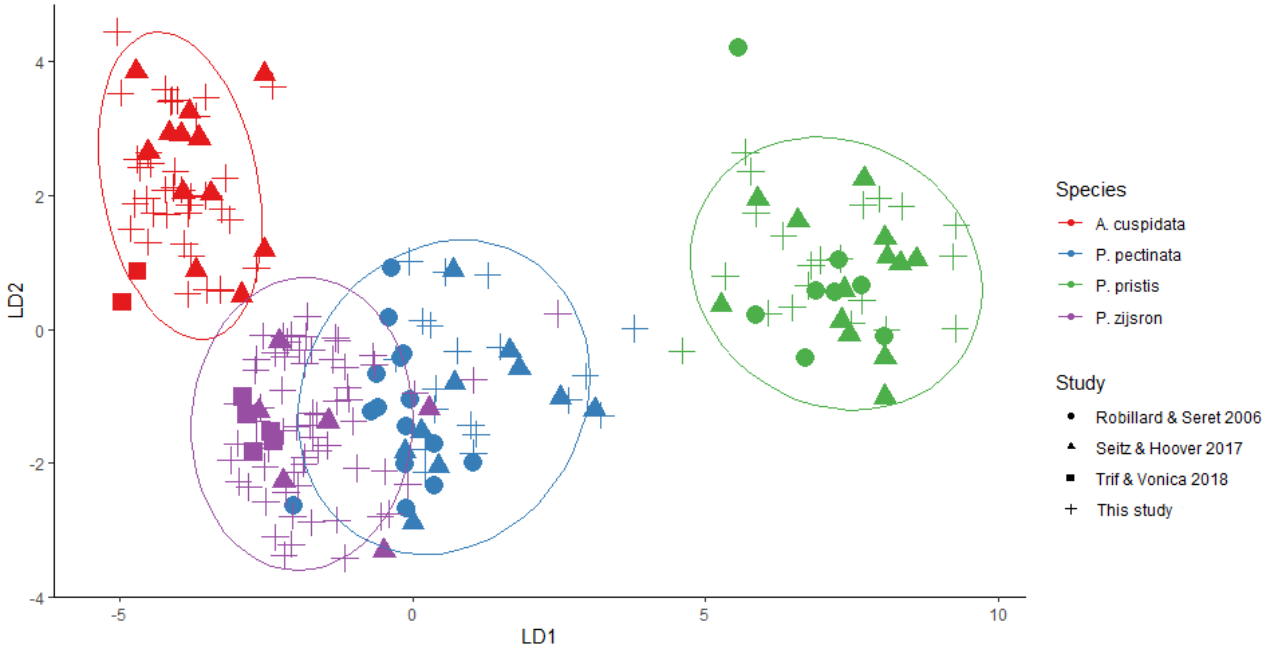

Figure 3 Results of the linear discriminant analysis (LDA), based on standardised measures of sawfish rostra. The first two discriminant functions account for 98.99% of the variance in the data.

Table 3 Prediction accuracy table (confusion matrix) of the four sawfish species. The numbers in brackets indicate the proportion of correct/incorrect predicted classification for each species.

	Predicted			
Actual	*A. cuspidata*	*P. pectinata*	*P. pristis*	*P. zijsron*
A. cuspidata	51 (100%)	0	0	0
P. pectinata	0	41 (93.18%)	0	3 (6.82%)
P. pristis	0	0	42 (100%)	0
P. zijsron	0	4 (5.41 %)	0	70 (94.59%)

Descriptive statistics of standardized measures were indicated for each species in Table 4, while all linear measures were listed and available in Table S4. The estimated total length (TL) ranged from 1,154 mm to 4,119 mm for *A. cuspidata*, from 881 mm to 3,800 mm for *P. pectinata*, from 777 mm to 6,826 mm for *P. pristis* and from 694 mm to 4,247 mm for *P. zijsron* (Table S4). In total, 49 rostra belonged to juvenile individuals, corresponding to a SRL < 289 mm (< 2,000 mm of estimated TL) for the species *A. cuspidata*, and with a SRL < 700 mm for *P. pectinata*, SRL < 617 mm for *P. pristis*, and < 840 mm for *P. zijsron* (estimated TL < 3,000 mm).

Table 4 Sample size, rostral variable ranges (given as percentages of TRL) and rostral tooth counts (teeth and empty alveoli) for each sawfish species of museum and private collections.

Species	N		sSRL	sPRW	sDRW	sPSD	sDSD	Nright	Nleft	Estimated TL (mm)
Anoxypristis cuspidata	39	Mean	78.50	8.38	4.92	4.15	1.07	25.82	25.61	2627.97
		St. Dev	4.84	0.76	0.77	0.58	0.25	2.79	2.67	468.24
		Min	68.75	6.94	4.00	3.03	0.69	17.00	16.00	1153.95
		Max	88.32	10.00	7.26	5.37	1.67	32.00	32.00	4119.21
Pristis pectinata	20	Mean	91.70	14.26	7.10	5.55	1.95	25.05	25.21	2369.52
		St. Dev	5.48	1.33	1.05	0.76	0.29	2.57	2.25	1128.10
		Min	72.99	12.33	5.05	3.97	1.54	20.00	22.00	881.27
		Max	97.62	17.02	8.89	6.93	2.67	30.00	31.00	3799.96
Pristis pristis	30	Mean	93.54	19.05	7.67	5.38	3.58	19.10	18.93	4565.67
		St. Dev	3.13	1.44	0.88	0.54	0.49	2.22	1.91	1427.67
		Min	85.45	16.24	6.07	4.24	2.46	15.00	15.00	777.13
		Max	99.12	21.94	9.66	6.42	4.57	24.00	24.00	6826.20
Pristis zijsron	80	Mean	91.24	10.93	5.39	6.19	1.14	29.82	29.78	3125.81
		St. Dev	3.20	1.26	1.15	0.98	0.33	2.61	2.71	1066.85
		Min	85.38	8.97	3.89	4.02	0.61	22.00	23.00	694.04
		Max	97.94	15.59	9.09	8.52	2.69	36.00	35.00	4247.09

Only 157 of the collected pictures were suitable for the digitalization of landmarks. The PCA with10 landmarks (Figure 4), showed a very clear separation among species with the principal component 1 (PC1) accounting for 84.89% of the total shape variation. The major movement in PC1 was with landmarks 6 and 7, showing a tendency toward a mostly distal and slightly inferior migration and, landmarks 8, 9 and 10 moving proximal-inferiorly (Figure S1). The Procrustes ANOVA performed among species showed statistically significant values (p-value <0,0001) both for the centroid size and the shape (Table 5).

The comparison of both morphometric approaches showed that 80 historical specimens belonged to the species *P. zijsron*, 39 to *A. cuspidata*, 30 to *P. pristis* and 20 to *P. pectinata*.

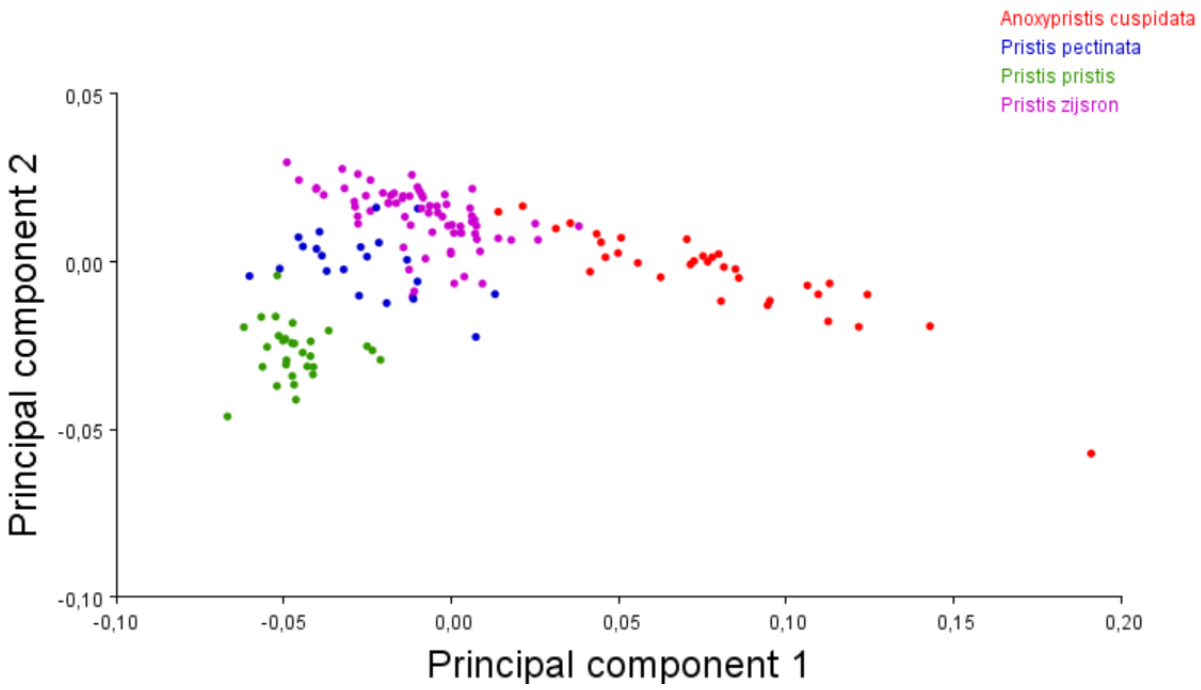

Figure 4 Principal Component Analysis based on landmark.

Table 5 Procrustes ANOVA testing for centroid size and shape differences among rostra of sawfish species.

Data	Factor	Sum of squares	Mean square	df	F	P
Centroid size	Individual	21991015.539	7330338.513	3	14.70	<.0001
	Residual	74788782.647	498591.884	150		
Shape	Individual	0.319	0.007	48	88.19	<.0001
	Residual	0.102	0.000	2400		

df, degrees of freedom; F, F statistic; P, associated probability level

2.4 Discussion

The global recovery of the remaining sawfish populations is undoubtedly an urgent need, involving considerable research to properly document the vulnerable status, declines, and disappearances of sawfish populations from many coastal areas around the oceans (Dulvy et al., 2016; Yan et al., 2021). Recently, huge research efforts are becoming more and more widespread to set appropriate targets for the recovery of sawfish populations, especially in Australia, for *P. pristis* (Whitty et al., 2017; Kyne et al., 2021), *P. clavata* (Morgan et al., 2021) and *P. zijsron* (Morgan et al., 2015; Morgan et al., 2017) and in Florida, for *P. pectinata* (Graham et al., 2021; Smith et al., 2021). Immediate actions are required to better assess the status of the species in poorly studied regions and to initiate biological studies providing the data needed to establish conservation and restoration plans. For these reasons, in recent years, numerous countries are putting efforts toward determining the conservation status of sawfish, such as United States (Seitz & Poulakis, 2006), Brazil (Reis-Filho et al., 2016), Guinea-Bissau (Leeney & Poncelet, 2015), countries of the Mediterranean (Ferretti et al., 2016), Red Sea countries (Elhassan, 2018) and Arabian Gulf (Jabado et al., 2017), Bangladesh (Haque & Das, 2019; Haque, Leeney, & Biswas, 2020) and Papua New Guinea (White et al., 2017).

The use of a multidisciplinary approach, with the combination of morphological and molecular analyses carried out on historical museum and private collections, represents a successful strategy to advance in the knowledge of taxonomy, systematics, and historical ecology of the sawfish populations. Historical data are important to understand the population dynamics of declining or locally extirpated populations and are of great utility for the conservation of relict populations living in other marine zoogeographic regions. It is nowadays established that combining molecular and morphological approaches can considerably improve the understanding of elasmobranch diversity (Lim et al., 2015; Last, Naylor, & Manjaji-Matsumoto, 2016). Molecular tools have become an essential approach for the identification of species, providing a systematic means to overcome the difficulties imposed by the absence of diagnostic morphological traits (Richards et al., 2009; Schluessel et al., 2010; Fontenelle et al., 2021).

In this study, the combined molecular-morphometric tools allowed the specific identification of 172 retrieved museum finds, assigned to four species: *Pristis zijsron*, *Anoxypristis cuspidata*, *P. pristis* and *P. pectinata*. The great improvement of our analyses was the noticeably increase in the

importance of the museum's exhibits, since 68 out of 172 rostra had not previously been identified at the species level and 34 rostra had been misidentified, mainly involving the species *P. zijsron*, previously erroneously labelled as *P. pectinata.* These two species appeared the most difficult to distinguish, as the rostra of these species were the most phenotypically similar. Nevertheless, the mtDNA-based species-specific identification of each adult individual was coherently confirmed by the morphological assignment. On the contrary, four rostra of juvenile individuals provided discordant identification being identified *P. zijsron* by mtDNA and *P. pectinata* by morphology. Most of the variables (i.e. measures and geometric shapes of rostra) used are correlated with rostrum length and become more distinct with growth, so they are less reliable in identifying juvenile *P. zijsron* (Whitty et al., 2014). Previous studies showed that a combination of rostral characters could discern and identify sawfish at the species level (Faria et al., 2013; Whitty et al., 2014; Seitz & Hoover, 2017). Faria et al. (2013) used canonical variate analysis and Whitty et al. (2014) used discriminant analysis. In both studies, species were identified beforehand. In contrast, Seitz & Hoover (2017) used PCA among individuals with no prior identification of species and verified the utility of rostral measurements as informative characters. Our dual approach allowed to be more confident in the morphological characterization. As a matter of fact, landmark-based analyses performed on rostrum exhibited a significant difference in terms of shape and centroid size between species, in accord with the traditional method. Morphometric studies based on landmarks were a useful tool for systematics, able to discriminate among different teleost species (Ibáñez & O'Higgins, 2011; Liotta et al., 2021), to quantify shape variation on elasmobranchs fins (Franklin, Palmer, & Dyke, 2014; Bellodi et al., 2022) and teeth (Adnet, 2006; Nyberg, Ciampaglio, & Wray, 2006; Whitenack & Gottfried, 2010).

The main challenge of the molecular tool applied to museum samples has been characterised by the degradation of DNA due to the exposure of the remains to the environment and oxidative stressors. Loss of nucleotides and fragmentation of large portions of the genome are characteristic of degraded DNA (Lindahl, 1993; Hofreiter et al., 2001; Pääbo et al., 2004). However, hard tissues generally tend to remain relatively stable over time (Anchordoquy & Molina, 2007) and the dried nature of the rostra seems to spare them from the rapid and extensive degradation that affects soft tissues (Wandeler et al., 2007; Phillips et al., 2009). Few studies included sequences generated from dried historical rostra of sawfish. The study of Phillips et al. (2009) was able to amplify 465-468 bp of the 12S gene marker, while Faria et al. (2013) amplified 148-480 bp of the mitochondrial NADH2 marker, reaching the maximum size range for DNA fragments in dried museums specimens. Our final alignments were about 350 bp and 320 bp in total length for NADH2 and COI datasets, respectively. All the species delimitation analyses carried out in this study required robust reference sequence datasets to produce

consistent results. The current lack of proper reference sequences for the comparison could be overcome as new reference sequences could be obtained from fresh tissues collected from all five sawfish species. The sampling of these fresh tissues could be performed from individuals held in aquariums to not damage the already endangered wild populations or sampling with available not invasive methods (e.g., mucus swab; Kashiwagi et al., 2015; Domingues et al., 2019). For the species *P. zijsron* only one NADH2 and three COI available sequences were retrieved from NCBI and BOLD, primarily from the Australian coasts, thus limiting further population structure analyses within the species.

The 16 samples labelled as Mediterranean origin deserve more attention: in particular, six individuals have been identified as *P. zijsron*, five as *A. cuspidata*, three as *P. pectinata* and one as *P. pristis*. Solely *P. pectinata* and *P. pristis* were the two species for which the occurrence in the Mediterranean basin was demonstrated based on original and independent records, until the second half of the last century (Ferretti et al., 2016). From literature, *A. cuspidata* may have inhabited the Mediterranean Sea in the past, with fossil records reported to the late Neogene and from the early Pliocene in Libya and Spain (Pawellek et al., 2012), and Tuscany (Collareta et al., 2017). No records are known from the literature concerning the sight or the catch of the species *P. zijsron* in the Mediterranean basin, in fact the species have a distribution range in the Indo-West Pacific Ocean, reaching the shores of the Red Sea (Dulvy et al., 2016). Most of the *P. zijsron* rostra labelled as Mediterranean referred to catches in the Adriatic Sea at the beginning of the past century.

In the light of what has been achieved, a rationale could be discussed trying to answer at the following three questions which point out the role of specimens labelled as Mediterranean origin, especially those belonging to non-historically Mediterranean species.

(1) Are the rostra really from the Mediterranean Sea or are they the result of the trade from other areas? The Mediterranean basin has always been in the past a crossroad for commercial trade between diverse and distant human populations, and sawfish rostra have been long exchanged among traders and collectors of natural history objects. The traffic of these distinctive and curious objects, often considered as trophies, was common for nearly two centuries (Norman & Fraser, 1938; Migdalski, 1960; Hoover, 2008). All the information related to the rostra museum finds can be useful and give suggestions about the history of the specimens. Two of the Mediterranean samples of the Natural History Museum of Rovereto (TN, Italy) carried on the label some more information about the origin site, that was the Genoa and Venice markets, respectively. These two markets are known to have been the most important markets in Italy in the 20th century, where exotic natural items were often exchanged and sold to private collectors (Antonio Di Natale, personal communication).

(2) Is it possible that *P. zijsron* occurred in the Mediterranean Sea? The description of this species is relatively recent (1851), while for *P. pectinata* the description dates to 1794. In the early 20th century, access to new publications was not as fast or easy as it is today. Thus, it is possible that past scientists identified the species in the Mediterranean according to taxonomic treaties available at that time, many of which could not have been updated with the latest classification of the group.

(3) Is it possible that such big species came through the Suez Canal? *Pristis zijsron* could have entered in the eastern Mediterranean from the Red Sea after the opening of the Suez Canal, a type of migration that is already documented for many other fish species (Lipej et al., 2017). The Suez Canal was officially opened in 1869 and was finally built to 163 km in length, a bottom of 22 m wide and 8 m deep. The first Lessepsian migrant fish in the Adriatic Sea was caught in Rijeka in 1896 (Dulčićet al., 2004).

The further Chapters 3 and 4 contribute to answer to these questions by using other integrated analytical approaches (e.g., stable isotope analysis). This multidisciplinary approach combined the species taxonomy of historical rostra together with the analysis of stable isotopes of carbon and oxygen in rostral teeth and the estimate of probability of the sample's likelihood to be assigned to the most probable geographical origin.

In conclusion, due to the current rarity of sawfishes in many areas and their conservation status, the museum collections of dried sawfish rostra have proved to be an effective and invaluable asset for a better understanding the natural history of these fascinating fishes, and the dual molecular and morphological approach effective tools to study these ancient and historical sawfish. Similar approaches using archaeological and historical skeletal remains have documented and traced back the natural and fishery histories of iconic fish as cod *Gadus morhua* (García, Lucifora, & Myers, 2008; Ólafsdóttir et al., 2014; Star et al., 2017), bluefin tuna *Thunnus thynnus* (Riccioni et al., 2010; Andrews et al., 2021, 2022a, b) and, among elasmobranchs, the great white shark *Carcharodon carcharias* (Leone et al., 2020).

However, the new knowledge provided by the present study require to be complemented with further studies on the structure of sawfish populations and on their evolutionary dynamics to identify a more suitable and efficient conservation policy.

2.5 References

Adams, D. C., Rohlf, F. J., & Slice, D. E. (2004). Geometric morphometrics: Ten years of progress following the 'revolution.' *Ital. J. Zool.* **71**, 5–16.

Adnet, S. (2006). Biometric analysis of the teeth of fossil and Recent hexanchid sharks and its taxonomic implications. *Acta Palaeontol. Pol.* **51**.

Allentoft, M. E., Sikora, M., Sjögren, K.-G., Rasmussen, S., Rasmussen, M., Stenderup, J., Damgaard, P. B., Schroeder, H., Ahlström, T., Vinner, L., Malaspinas, A.-S., Margaryan, A., Higham, T., Chivall, D., Lynnerup, N., Harvig, L., Baron, J., Casa, P. D., Dąbrowski, P., Duffy, P. R., Ebel, A. V., Epimakhov, A., Frei, K., Furmanek, M., Gralak, T., Gromov, A., Gronkiewicz, S., Grupe, G., Hajdu, T., Jarysz, R., Khartanovich, V., Khokhlov, A., Kiss, V., Kolář, J., Kriiska, A., Lasak, I., Longhi, C., McGlynn, G., Merkevicius, A., Merkyte, I., Metspalu, M., Mkrtchyan, R., Moiseyev, V., Paja, L., Pálfi, G., Pokutta, D., Pospieszny, Ł., Price, T. D., Saag, L., Sablin, M., Shishlina, N., Smrčka, V., Soenov, V. I., Szeverényi, V., Tóth, G., Trifanova, S. V., Varul, L., Vicze, M., Yepiskoposyan, L., Zhitenev, V., Orlando, L., Sicheritz-Pontén, T., Brunak, S., Nielsen, R., Kristiansen, K., & Willerslev, E. (2015). Population genomics of Bronze Age Eurasia. *Nature* **522**, 167–172.

Anchordoquy, T. J., & Molina, M. C. (2007). Preservation of DNA. *Cell Preserv. Technol.* **5**, 180–188.

Andrews, A. J., Di Natale, A., Bernal-Casasola, D., Aniceti, V., Onar, V., Oueslati, T., Theodropoulou, T., Morales-Muñiz, A., Cilli, E., & Tinti, F. (2022). Exploitation history of Atlantic bluefin tuna in the eastern Atlantic and Mediterranean insights from ancient bones. *ICES J. Mar. Sci*, **0**, 1-18.

Andrews, A. J., Mylona, D., Rivera, L., Winter, R., Onar, V., Siddiq, A. B., Tinti, F., & Morales-Muniz, A. (2022). Length estimation of Atlantic bluefin tuna (*Thunnus thynnus*) using vertebrae. *Int. J. Osteoarchaeol.*

Andrews, A. J., Puncher, G. N., Bernal-Casasola, D., Di Natale, A., Massari, F., Onar, V., Toker, N. Y., Hanke, A., Pavey, S. A., Savojardo, C., Martelli, P. L., Casadio, R., Cilli, E., Morales-Muñiz, A., Mantovani, B., Tinti, F., & Cariani, A. (2021). Ancient DNA SNP-panel data suggests stability in bluefin tuna genetic diversity despite centuries of fluctuating catches in the eastern Atlantic and Mediterranean. *Sci. Rep.* **11**, 20744.

Cabanillas-Torpoco, M., Castillo, D., Siccha-Ramírez, R., Forsberg, K., Purizaca, W., & Maceda, M. (2020). Occurrence of the largetooth sawfish Pristis pristis (Linneaus, 1758) in northern Peru. *Zootaxa* **4868**, 147–150.

Carlson, J. K., Wiley, T., & Smith, K. (2013). IUCN Red List of Threatened Species: Pristis pectinata. *IUCN Red List Threat. Species*.

Collins, R. A., & Cruickshank, R. H. (2013). The seven deadly sins of DNA barcoding. *Mol. Ecol. Resour.* **13**, 969–975.

Compagno, L. J. V., & Last, P. R. (1999). Family Pristidae: Sawfish. *FAO Identif. Guide Fish. Purp. Living Mar. Resour. West. Cent. Pac. FAO Rome*.

Cooper, A., & Poinar, H. N. (2000). Ancient DNA: Do It Right or Not at All. *Science* **289**, 1139–1139.

D'Anastasi, B., Simpfendorfer, C., & van Herwerden, L. (2013). IUCN Red List of Threatened Species: Anoxypristis cuspidata. *IUCN Red List Threat. Species*.

Dabney, J., Meyer, M., & Pääbo, S. (2013). Ancient DNA Damage. *Cold Spring Harb. Perspect. Biol.* **5**, a012567.

Dulčić, J., Pallaoro, A., 2004. First record of the marbled spinefoot, Siganus rivulatus (Pisces, Siganidae) in the Adriatic Sea. *J. Mar. Biolog. Assoc.* **84**, 1087-1088.

Dulvy, N. K., Davidson, L. N. K., Kyne, P. M., Simpfendorfer, C. A., Harrison, L. R., Carlson, J. K., & Fordham, S. V. (2016). Ghosts of the coast: global extinction risk and conservation of sawfishes. *Aquat. Conserv. Mar. Freshw. Ecosyst.* **26**, 134–153.

Dulvy, N. K., Fowler, S. L., Musick, J. A., Cavanagh, R. D., Kyne, P. M., Harrison, L. R., Carlson, J. K., Davidson, L. N. K., Fordham, S. V., Francis, M. P., Pollock, C. M., Simpfendorfer, C. A., Burgess, G. H., Carpenter, K. E., Compagno, L. J. V., Ebert, D. A., Gibson, C., Heupel, M. R., Livingstone, S. R., Sanciangco, J. C., Stevens, J. D., Valenti, S., & White, W. T. (2014). Extinction risk and conservation of the world's sharks and rays. *eLife*. **3**, e00590.

Dulvy, N. K., Pacoureau, N., Rigby, C. L., Pollom, R. A., Jabado, R. W., Ebert, D. A., Finucci, B., Pollock, C. M., Cheok, J., Derrick, D. H., Herman, K. B., Sherman, C. S., VanderWright, W. J., Lawson, J. M., Walls, R. H. L., Carlson, J. K., Charvet, P., Bineesh, K. K., Fernando, D., Ralph, G. M., Matsushiba, J. H., Hilton-Taylor, C., Fordham, S. V., & Simpfendorfer, C. A.

(2021). Overfishing drives over one-third of all sharks and rays toward a global extinction crisis. *Curr. Biol.* **31**, 1–15.

Elhassan, I. S. (2018). Occurrence of the green sawfish *Pristis zijsron* in the Sudanese Red Sea with observations on reproduction. *Endanger. Species Res.* **36**, 41–47.

Faria, V. V., McDavitt, M. T., Charvet, P., Wiley, T. R., Simpfendorfer, C. A., & Naylor, G. J. P. (2013). Species delineation and global population structure of Critically Endangered sawfishes (Pristidae). *Zool. J. Linn. Soc.* **167**, 136–164.

Felsenstein, J. (1985). Confidence Limits on Phylogenies: An Approach Using the Bootstrap. *Evolution* **39**, 783–791.

Fontenelle, J. P., Lovejoy, N. R., Kolmann, M. A., & Marques, F. P. L. (2021). Molecular phylogeny for the Neotropical freshwater stingrays (Myliobatiformes: Potamotrygoninae) reveals limitations of traditional taxonomy. *Biol. J. Linn. Soc.* **134**, 381–401.

Franklin, O., Palmer, C., & Dyke, G. (2014). Pectoral fin morphology of batoid fishes (Chondrichthyes: Batoidea): Explaining phylogenetic variation with geometric morphometrics. *J. Morphol.* **275**, 1173–1186.

Fulton, T. L. (2012). Setting Up an Ancient DNA Laboratory. In B. Shapiro & M. Hofreiter (Eds.), *Anc. DNA Methods Protoc.* pp. 1–11. Totowa, NJ: Humana Press.

García, V. B., Lucifora, L. O., & Myers, R. A. (2008). The importance of habitat and life history to extinction risk in sharks, skates, rays and chimaeras. *Proc. R. Soc. B Biol. Sci.* **275**, 83–89.

Gilbert, M. T. P., Bandelt, H.-J., Hofreiter, M., & Barnes, I. (2005). Assessing ancient DNA studies. *Trends Ecol. Evol.* **20**, 541–544.

Graham, J., Kroetz, A. M., Poulakis, G. R., Scharer, R. M., Carlson, J. K., Lowerre-Barbieri, S., Morley, D., Reyier, E. A., & Grubbs, R. D. (2021). Large-scale space use of large juvenile and adult smalltooth sawfish Pristis pectinata: implications for management. *Endanger. Species Res.* **44**, 45–59.

Guindon, S., Dufayard, J.-F., Lefort, V., Anisimova, M., Hordijk, W., & Gascuel, O. (2010). New Algorithms and Methods to Estimate Maximum-Likelihood Phylogenies: Assessing the Performance of PhyML 3.0. *Syst. Biol.* **59**, 307–321.

Haque, A. B., & Das, S. A. (2019). First confirmed record of the Critically Endangered green sawfish *Pristis zijsron* from Bangladeshi waters. *J. Fish Biol.* **94**, 200–203.

Haque, A. B., Leeney, R. H., & Biswas, A. R. (2020). Publish, then perish? Five years on, sawfishes are still at risk in Bangladesh. *Aquat. Conserv. Mar. Freshw. Ecosyst.* **30**, 2370–2383.

Harrison, L. R., & Dulvy, N. K. (2014). Sawfish: a global strategy for conservation. IUCN.

Hofreiter, M., Serre, D., Poinar, H. N., Kuch, M., & Pääbo, S. (2001). ancient DNA. *Nat. Rev. Genet.* **2**, 353–359.

Ibáñez, A. L., & O'Higgins, P. (2011). Identifying fish scales: The influence of allometry on scale shape and classification. *Fish. Res.* **109**, 54–60.

Jabado, R., Al Baharna, R., Al Ali, S., Al Suwaidi, K., Al Blooshi, A., & Al Dhaheri, S. (2017). Is this the last stand of the Critically Endangered green sawfish *Pristis zijsron* in the Arabian Gulf? *Endanger. Species Res.* **32**, 265–275.

Klingenberg, C. P. (2011). MorphoJ: an integrated software package for geometric morphometrics. *Mol. Ecol. Resour.* **11**, 353–357.

Klingenberg, C. P., & McIntyre, G. S. (1998). Geometric Morphometrics of Developmental Instability: Analyzing Patterns of Fluctuating Asymmetry with Procrustes Methods. *Evolution* **52**, 1363–1375.

Klingenberg, C. P., Barluenga, M., & Meyer, A. (2002). Shape Analysis of Symmetric Structures: Quantifying Variation Among Individuals and Asymmetry. *Evolution* **56**, 1909–1920.

Kumar, S., Stecher, G., & Tamura, K. (2016). MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Mol. Biol. Evol.* **33**, 1870–1874.

Kyne, P. M., Carlson, J., & Smith, K. (2013). IUCN Red List of Threatened Species: Pristis pristis. *IUCN Red List Threat. Species*.

Kyne, P. M., Oetinger, M., Grant, M. I., & Feutry, P. (2021). Life history of the Critically Endangered largetooth sawfish: a compilation of data for population assessment and demographic modelling. *Endanger. Species Res.* **44**, 79–88.

Kyne, P., Rigby, C., & Simpfendorfer, C. (2013). IUCN Red List of Threatened Species: Pristis clavata. *IUCN Red List Threat. Species*.

Last, P. R., Naylor, G. J., & Manjaji-Matsumoto, B. M. (2016). A revised classification of the family Dasyatidae (Chondrichthyes: Myliobatiformes) based on new morphological and molecular insights. *Zootaxa* **4139**, 345–368.

Last, P. R., Stevens, J. D., Swainston, R., & Davis, G. (2009). Sharks and rays of Australia.

Leone, A., Puncher, G. N., Ferretti, F., Sperone, E., Tripepi, S., Micarelli, P., Gambarelli, A., Sarà, M., Arculeo, M., Doria, G., Garibaldi, F., Bressi, N., Dall'Asta, A., Minelli, D., Cilli, E., Vanni, S., Serena, F., Díaz-Jaimes, P., Baele, G., Cariani, A., & Tinti, F. (2020). Pliocene colonization of the Mediterranean by Great White Shark inferred from fossil records, historical jaws, phylogeographic and divergence time analyses. *J. Biogeogr.* **47**, 1119–1129.

Lim, K. C., Lim, P.-E., Chong, V. C., & Loh, K.-H. (2015). Molecular and Morphological Analyses Reveal Phylogenetic Relationships of Stingrays Focusing on the Family Dasyatidae (Myliobatiformes). *PLOS ONE* **10**, e0120518.

Lindahl, T. (1993). Instability and decay of the primary structure of DNA. *nature* **362**, 709–715.

Liotta, M. N., Abbott, J. K., Morris, M. R., & Rios-Cardenas, O. (2021). Antagonistic selection on body size and sword length in a wild population of the swordtail fish, Xiphophorus multilineatus: Potential for intralocus tactical conflict. *Ecol. Evol.* **11**, 3941–3955.

Llamas, B., Valverde, G., Fehren-Schmitz, L., Weyrich, L. S., Cooper, A., & Haak, W. (2017). From the field to the laboratory: Controlling DNA contamination in human ancient DNA research in the high-throughput sequencing era. *STAR Sci. Technol. Archaeol. Res.* **3**, 1–14.

Mace, G. M. (2004). The role of taxonomy in species conservation. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* **359**, 711–719.

Melo Palmeira, C. A., da Silva Rodrigues-Filho, L. F., de Luna Sales, J. B., Vallinoto, M., Schneider, H., & Sampaio, I. (2013). Commercialization of a critically endangered species (largetooth sawfish, Pristis perotteti) in fish markets of northern Brazil: Authenticity by DNA analysis. *Food Control* **34**, 249–252.

Moftah, M., Aziz, S. H. A., Elramah, S., & Favereaux, A. (2011). Classification of Sharks in the Egyptian Mediterranean Waters Using Morphological and DNA Barcoding Approaches. *PLOS ONE* **6**, e27001.

Morgan, D. L., Allen, M. G., Ebner, B. C., Whitty, J. M., & Beatty, S. J. (2015). Discovery of a pupping site and nursery for critically endangered green sawfish Pristis zijsron. *J. Fish Biol.* **86**, 1658–1663.

Morgan, D. L., Ebner, B. C., Allen, M. G., Gleiss, A. C., Beatty, S. J., & Whitty, J. M. (2017). Habitat use and site fidelity of neonate and juvenile green sawfish Pristis zijsron in a nursery area in Western Australia. *Endanger. Species Res.* **34**, 235–249.

Morgan, D. L., Lear, K. O., Dobinson, E., Gleiss, A. C., Fazeldean, T., Pillans, R. D., Beatty, S. J., & Whitty, J. M. (2021). Seasonal use of a macrotidal estuary by the endangered dwarf sawfish, Pristis clavata. *Aquat. Conserv. Mar. Freshw. Ecosyst.* **31**, 2164–2177.

Morgan, D. L., Whitty, J. M., Phillips, N. M., Thorburn, D. C., Chaplin, J. A., & McAuley, R. (2011). North-western Australia as a hotspot for endangered elasmobranchs with particular reference to sawfishes and the Northern River Shark. *J. R. Soc. Wes.t Aus.*, 94(2), 345-358.

Nyberg, K. G., Ciampaglio, C. N., & Wray, G. A. (2006). Tracing the ancestry of the great white shark, Carcharodon carcharias, using morphometric analyses of fossil teeth. *J. Vertebr. Paleontol.* **26**, 806–814.

Ólafsdóttir, G. Á., Westfall, K. M., Edvardsson, R., & Pálsson, S. (2014). Historical DNA reveals the demographic history of Atlantic cod (Gadus morhua) in medieval and early modern Iceland. *Proc. R. Soc. B Biol. Sci.* **281**, 20132976.

Pääbo, S. (1989). Ancient DNA: extraction, characterization, molecular cloning, and enzymatic amplification. *Proc. Natl. Acad. Sci.* **86**, 1939–1943.

Pääbo, S., Poinar, H., Serre, D., Jaenicke-Després, V., Hebler, J., Rohland, N., Kuch, M., Krause, J., Vigilant, L., & Hofreiter, M. (2004). Genetic Analyses from Ancient DNA. *Annu. Rev. Genet.* **38**, 645–679.

Petean, F. F., Naylor, G. J. P., & Lima, S. M. Q. (2020). Integrative taxonomy identifies a new stingray species of the genus Hypanus Rafinesque, 1818 (Dasyatidae, Myliobatiformes), from the Tropical Southwestern Atlantic. *J. Fish Biol.* **97**, 1120–1142.

Phillips, N., Chaplin, J., Morgan, D., & Peverell, S. (2009). Extraction and amplification of DNA from the dried rostra of sawfishes (Pristidae) for applications in conservation genetics. *Pac. Conserv. Biol.* **15**, 128–134.

Puillandre, N., Brouillet, S., & Achaz, G. (2021). ASAP: assemble species by automatic partitioning. *Mol. Ecol. Resour.* **21**, 609–620.

Puncher, G. N., Cariani, A., Cilli, E., Massari, F., Leone, A., Morales-Muñiz, A., Onar, V., Toker, N. Y., Casasola, D. B., Moens, T., & Tinti, F. (2019). Comparison and optimization of genetic tools used for the identification of ancient fish remains recovered from archaeological excavations and museum collections in the Mediterranean region. *Int. J. Osteoarchaeol.* **29**, 365–376.

R Core Team (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Reis-Filho, J. A., Freitas, R. H. A., Loiola, M., Leite, L., Soeiro, G., Oliveira, H. H. Q., Sampaio, C. L. S., Nunes, J. de A. C. C., & Leduc, A. O. H. C. (2016). Traditional fisher perceptions on the regional disappearance of the largetooth sawfish *Pristis pristis* from the central coast of Brazil. *Endanger. Species Res.* **29**, 189–200.

Riccioni, G., Landi, M., Ferrara, G., Milano, I., Cariani, A., Zane, L., Sella, M., Barbujani, G., & Tinti, F. (2010). Spatio-temporal population structuring and genetic diversity retention in depleted Atlantic Bluefin tuna of the Mediterranean Sea. *Proc. Natl. Acad. Sci.* **107**, 2102–2107.

Richards, V. P., Henning, M., Witzell, W., & Shivji, M. S. (2009). Species Delineation and Evolutionary History of the Globally Distributed Spotted Eagle Ray (Aetobatus narinari). *J. Hered.* **100**, 273–283.

Robillard, M., & Séret, B. (2006). Cultural importance and decline of sawfish (Pristidae) populations in West Africa 8.

Rohlf, F.J. (2006). tpsDig, version 2.10. Stony Brook, NY: Department of Ecology and Evolution, State University of New York.

Rohlf, F. J. (2015). The tps series of software. *Hystrix Ital. J. Mammal.* **26**, 9–12.

Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D. L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M. A., & Huelsenbeck, J. P. (2012). MrBayes 3.2: Efficient Bayesian Phylogenetic Inference and Model Choice Across a Large Model Space. *Syst. Biol.* **61**, 539–542.

Saitou, N., & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* **4**, 406–425.

Schluessel, V., Broderick, D., Collin, S. P., & Ovenden, J. R. (2010). Evidence for extensive population structure in the white-spotted eagle ray within the Indo-Pacific inferred from mitochondrial gene sequences. *J. Zool.* **281**, 46–55.

Seitz, J. C., & Hoover, J. J. (2017). Taxonomic resolution of sawfish rostra from two private collections. *Endanger. Species Res.* **32**, 525–532.

Serventi, P., Panicucci, C., Bodega, R., Fanti, S. D., Sarno, S., Alvarez, M. F., Brisighelli, F., Trombetta, B., Anagnostou, P., Ferri, G., Vazzana, A., Delpino, C., Gruppioni, G., Luiselli, D., & Cilli, E. (2018). Iron Age Italic population genetics: the Piceni from Novilara (8th–7th century BC). *Ann. Hum. Biol.* **45**, 34–43.

Simpfendorfer, C. (2013). IUCN Red List of Threatened Species: Pristis zijsron. *IUCN Red List Threat. Species*.

Simpfendorfer, C. A., & Dulvy, N. K. (2017). Bright spots of sustainable shark fishing. *Curr. Biol.* **27**, R97–R98.

Smith, K. L., Feldheim, K., Carlson, J. K., Wiley, T. R., & Taylor, S. S. (2021). Female philopatry in smalltooth sawfish Pristis pectinata: conservation and management implications. *Endanger. Species Res.* **45**, 85–98.

Star, B., Boessenkool, S., Gondek, A. T., Nikulina, E. A., Hufthammer, A. K., Pampoulie, C., Knutsen, H., André, C., Nistelberger, H. M., Dierking, J., Petereit, C., Heinrich, D., Jakobsen, K. S., Stenseth, N. C., Jentoft, S., & Barrett, J. H. (2017). Ancient DNA reveals the Arctic origin of Viking Age cod from Haithabu, Germany. *Proc. Natl. Acad. Sci.* **114**, 9152–9157.

Stöver, B. C., & Müller, K. F. (2010). TreeGraph 2: Combining and visualizing evidence from different phylogenetic analyses. *BMC Bioinformatics* **11**.

Thompson, J. D., Higgins, D. G., & Gibson, T. J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.* **22**, 4673–4680.

Thorburn, D. C., Morgan, D. L., Rowland, A. J., Gill, H. S., & Paling, E. (2008). Life history notes of the critically endangered dwarf sawfish, Pristis clavata, Garman 1906 from the Kimberley region of Western Australia. *Environ. Biol. Fishes* **83**, 139–145.

Trif, N., & Vonica, G. (2018). Reassessment of the sawfish rostra taxonomy from the Natural History Museum in Sibiu. *Brukenthal Acta Musei* **13**, 517–530.

Venables, W. N., & Ripley, B. D. (2002). Modern Applied Statistics with S. 4th ed. New York: Springer-Verlag.

Wandeler, P., Hoeck, P. E. A., & Keller, L. F. (2007). Back to the future: museum specimens in population genetics. *Trends Ecol. Evol.* **22**, 634–642.

Whitenack †, L. B., & Gottfried, M. D. (2010). A morphometric approach for addressing tooth-based species delimitation in fossil mako sharks, Isurus (Elasmobranchii: Lamniformes). *J. Vertebr. Paleontol.* **30**, 17–25.

Whitty, J. M., Keleher, J., Ebner, B. C., Gleiss, A. C., Simpfendorfer, C. A., & Morgan, D. L. (2017). Habitat use of a Critically Endangered elasmobranch, the largetooth sawfish Pristis pristis, in an intermittently flowing riverine nursery. *Endanger. Species Res.* **34**, 211–227.

Whitty, J. M., Phillips, N. M., Thorburn, D. C., Simpfendorfer, C. A., Field, I., Peverell, S. C., & Morgan, D. L. (2014). Utility of rostra in the identification of Australian sawfishes (Chondrichthyes: Pristidae). *Aquat. Conserv. Mar. Freshw. Ecosyst.* **24**, 791–804.

Wueringer, B. E. (2012). Electroreception in Elasmobranchs: Sawfish as a Case Study. *Brain. Behav. Evol.* **80**, 97–107.

Wueringer, B. E., Squire, L., & Collin, S. P. (2009). The biology of extinct and extant sawfish (Batoidea: Sclerorhynchidae and Pristidae). *Rev. Fish Biol. Fish.* **19**, 445.

Zhang, J., Kapli, P., Pavlidis, P., & Stamatakis, A. (2013). A general species delimitation method with applications to phylogenetic placements. *Bioinforma. Oxf. Engl.* **29**, 2869–2876.

.

2.6 Supplementary material

Table S1 List of available public sequences used as reference dataset.

Species	Markers	N	Repository	Accession number (GenBank)	Process (BOLD)	Ocean	Ref
Anoxypristis cuspidata	COI	8	BOLD		SOPNG057-18, SOPNG067-18, SOPNG068-18, SOPNG117-18, SOPNG118-18, SOPNG120-18 - SOPNG121-18, SOPNG132-18	W Pacific Ocean	Ward et al., 2008
	COI	5	GenBank/ BOLD	EU398523 - EU398527	FOAF026-07-FOAF030-07	W Pacific Ocean	Ward et al., 2008
	Mitochondrial genome	2	GenBank/ BOLD	KP233202, NC_026307	GBMNA14188-19, GBMTG3749-16	W Pacific Ocean	Chen et al., 2015
	ND2	2	GenBank	JN184261, JQ518858		W Pacific Ocean	Aschliman et al., 2012; Naylor et al., 2012
Pristis clavata	COI	3	BOLD		FOAF031-07 IRREK892-08, KERRI357-09	W Pacific Ocean	
	COI	4	GenBank/ BOLD	EU398986 - EU398988, JN184072	FOAF147-07 - FOAF149-07, ANGBF2047-12	W Pacific Ocean	Ward et al., 2008; Aschliman et al., 2012
	Mitochondrial genome	2	GenBank/ BOLD	KF381507, NC_022821	GBMNA14189-19, GBMTG5128-16	W Pacific Ocean	Chen et al., 2015; Feutry et al., 2013
Pristis microdon	COI	2	BOLD		KERRI597-07, KERRI598-07	W Pacific Ocean	Robert Hanner
	ND2	2	GenBank	JN184262; JQ518861		W Pacific Ocean	Aschliman et al., 2012; Naylor et al., 2012
Pristis pectinata	COI	18	BOLD		IRREK497 - IRREK593; IRREK901; IRREK942 - IRREK950; PHANT412	W Atlantic Ocean	
	Mitochondrial genome	3	GenBank/ BOLD	KP400584; MF682494; NC_027182	GBMNA14190-19; GBMNA17986-19; GBMTG4504-16	W Atlantic Ocean	Chen et al., 2015; Díaz Jaimes et al., 2018
	ND2	2	GenBank	JN184263; JQ518859		W Atlantic Ocean	Aschliman et al., 2012; Naylor et al., 2012
Pristis perotteti	ND2	1	GenBank	JQ518860		W Atlantic Ocean	Naylor et al., 2012
Pristis pristis	COI	10	BOLD		FOAI360-09; SOPNG041; SOPNG065; SOPNG066; SOPNG069; SOPNG092; SOPNG107; SOPNG110; SOPNG114; SOPNG144	W Pacific Ocean	Ward et al., 2008

	COI	14	GenBank	MF977764; MN105755; MN105758 - MN105767; MN105840; MN105841		W Atlantic Ocean	Rodrigues Filho et al., 2020
	COI	4	GenBank/ BOLD	MH825681; EU398989; MH005928; NC_039438	GBGC18117-19; FOAF032-07; GBMNA18655-19; GBMNA18665-19	E Indian Ocean	Das & Haque 2018; Ward et al., 2008; Feutry et al., 2018
	ND2	1				Mediterranean Sea	Unpublic; G.Naylor GN4018_BMNH
Pristis zijsron	COI	2	BOLD		KERRI356-08, PHANT464	E Indian Ocean	Ward et al., 2008; Robert Hanner
	COI	1	GenBank/ BOLD	HM422390	FOAI415-09	W Indian Ocean	Ward et al., 2008
	ND2	1	GenBank	JQ519151		E Indian Ocean	Naylor et al., 2012

Table S2 List of primer developed in this study with specific details on melting (Tm) and annealing temperature (Ta) and product length.

Marker	name	Sequence (5'->3')	Tm	Ta °C	Product length
NADH2	SawND2_1F	ACCATAGCCATCATCCCATT	56.4	50	180
	SawND2_1R	GTGTGGCAGAGACTGGGTTT	60.5		
	SawND2_2F	TCTATTTGCCGGAACAACAA	54.3	50	212
	SawND2_2R	ATTGCGAATGGTGCAAGTTT	54.3		
	AnoxyND2_1F	TTTGATTTTCGGTGCTTGAG	54.81	50	202
	AnoxyND2_1R	TATAAGGGGAACCAGTCAGT	54.82		
	AnoxyND2_2F	AATGTAATTGTAACCGCCCA	55.32	50	193
	AnoxyND2_2R	CAACTCCAGCAGAGGTTAATA	55.03		
COI	SawCOI_1F	CTTGAGCAGGAATGGTTGGT	58.4	52	195
	SawCOI_1R	GCACCAATCATCAGAGGAACC	61.3		
	SawCOI_2F	TTGTAACCGCCCATGCCTTT	58.4	52	165
	SawCOI_2R	CGTGGGCAAGGTTACCAG	58.4		
	AnoxyCOI_1F	TAGGACTAGGCACCACAATC	56.33	50	180
	AnoxyCOI_1R	CAAATAAGAGAAGTGCCGAG	54.14		
	AnoxyCOI_2F	AGCCTCGGCACTTCTCTTAT	58.51	52	218
	AnoxyCOI_2R	GTGCAAGTTTTTGTCATGTGG	56.8		
	PectCOI_1F	GTGCTTGAGCAGGAATGGTTG	60.07	55	187
	PectCOI_1R	AGGGGAACCAGTCAGTTACCA	60.42		
	PectCOI_2F	TGGTAACTGACTGGTTCCCCTA	60.16	55	190
	PectCOI_2R	AGCTCCAGCATGAGCAAGATT	60.06		

Table S3 Details of the 172 individual specimens analysed: museum or private collections, geographical origin, location, year of sampling, and results of specific identification.

Museum	N	Museum label	Code_ Gen	Geographical Origin	Location	Year	Museum species	Species Morpho ID	Species Gen ID	DEF
Natural history museum of Trieste	49	Ic 3099	T26	Indian Ocean	Madras, India	1886	*Pristis pristis*	*P. pristis*	*P. pristis*	*P. pristis*
		Ic 3101	T27	-	-	-	*Pristis pristis*	*P. pristis*	*P. pristis*	*P. pristis*
		Ic 3105	T28	-	-	-	*Pristis pectinata*	*P. zijsron*	*P. zijsron*	*P. zijsron*
		Ic 3113	T29	-	-	-	*Pristis pectinata*	*P. zijsron*	*P. zijsron*	*P. zijsron*
		Ic 3108	T30	-	-	-	*Pristis pectinata*	*P. zijsron*	*P. zijsron*	*P. zijsron*
		Ic 3110	T31	-	-	-	*Pristis pectinata*	*P. zijsron*	*P. zijsron*	*P. zijsron*
		Ic 3111	T32	-	-	-	*Pristis pectinata*	*P. zijsron*	*P. zijsron*	*P. zijsron*
		Ic 3100	T33	-	-	-	*Pristis pristis*	*P. pristis*	*P. pristis*	*P. pristis*
		Ic 3106	T34	-	-	-	*Pristis pectinata*	*P. pectinata*	*P. pectinata*	*P. pectinata*
		Ic 3109	T35	-	-	-	*Pristis pectinata*	*P. zijsron*	*P. zijsron*	*P. zijsron*
		Ic 3107	T36	-	-	-	*Pristis pectinata*	*P. zijsron*	*P. zijsron*	*P. zijsron*
		Ic 3102	T37	-	-	-	*Pristis pristis*	*P. pristis*	*P. pristis*	*P. pristis*
		Ic 3085	T38	-	-	-	*Anoxypristis cuspidata*	*A. cuspidata*	*A. cuspidata*	*A. cuspidata*
		Ic 3104	T39	-	-	-	*Pristis pectinata*	*P. zijsron*	*P. zijsron*	*P. zijsron*
		Ic 3086	T40	-	-	1908	*Anoxypristis cuspidata*	*A. cuspidata*	*A. cuspidata*	*A. cuspidata*
		Ic 3087	T41	-	-	-	*Anoxypristis cuspidata*	*A. cuspidata*	*A. cuspidata*	*A. cuspidata*
		Ic 3112	T42	-	-	-	*Pristis pectinata*	*P. zijsron*	*P. zijsron*	*P. zijsron*
		Ic 3097	T43	-	-	-	*Anoxypristis cuspidata*	*A. cuspidata*	*A. cuspidata*	*A. cuspidata*
		Ic 3088	T44	-	-	-	*Anoxypristis cuspidata*	*A. cuspidata*	*A. cuspidata*	*A. cuspidata*
		Ic 3090	T45	-	-	-	*Anoxypristis cuspidata*	*A. cuspidata*	*A. cuspidata*	*A. cuspidata*
		Ic 3089	T46	-	-	-	*Anoxypristis cuspidata*	*A. cuspidata*	*A. cuspidata*	*A. cuspidata*
		Ic 3091	T47	-	-	-	*Anoxypristis cuspidata*	*A. cuspidata*	*A. cuspidata*	*A. cuspidata*
		Ic 3092	T48	-	-	-	*Anoxypristis cuspidata*	*A. cuspidata*	*A. cuspidata*	*A. cuspidata*
		Ic 3093	T49	-	-	-	*Anoxypristis cuspidata*	*A. cuspidata*	*A. cuspidata*	*A. cuspidata*
		Ic 2826	T50	Indian Ocean	Madras, India	1889	*Pristis pristis*	*P. pristis*	*P. pristis*	*P. pristis*
		Ic 2827	T51	West Pacific Ocean	Singapore	-	*Pristis pristis*	*P. pristis*	*P. pristis*	*P. pristis*
		Ic 2931	T52	-	-	1889	*Anoxypristis cuspidata*	*A. cuspidata*	*A. cuspidata*	*A. cuspidata*

		Ic 2932	T53	-	-	1889	*Pristis pectinata*	*P. zijsron*	*P. zijsron*	*P. zijsron*
		Ic 2933	T54	-	-	-	*Pristis pectinata*	*P. zijsron*	*P. zijsron*	*P. zijsron*
		Ic 2934	T55	-	-	-	*Anoxypristis cuspidata*	*A. cuspidata*	*A. cuspidata*	*A. cuspidata*
		Ic 2935	T56	-	-	-	*Pristis pectinata*	*P. zijsron*	*P. zijsron*	*P. zijsron*
		Ic 3114	T111	-	-	-	*Pristis zijsron*	*P. zijsron*	*P. zijsron*	*P. zijsron*
		Ic 3160	T112	-	-	-	*Pristis pristis*	*P. pristis*	*P. pristis*	*P. pristis*
		Ic 3161	T113	-	-	-	*Pristis pectinata*	*P. zijsron*	*P. zijsron*	*P. zijsron*
		Ic 3103	T114	-	-	-	*Pristis spp.*	*P. pectinata*	*P. zijsron*	*P. zijsron*
		Ic 3070	T115	-	-	-	*Pristis spp.*	*P. zijsron*	*P. zijsron*	*P. zijsron*
		Ic 3162	T116	-	-	-	*Pristis spp.*	*P. pectinata*	*P. zijsron*	*P. zijsron*
		Ic 3069	T117	-	-	-	*Anoxypristis cuspidata*	*A. cuspidata*	*A. cuspidata*	*A. cuspidata*
		Ic 3094	T118	-	-	-	*Anoxypristis cuspidata*	*A. cuspidata*	*A. cuspidata*	*A. cuspidata*
		Ic 3155	T119	-	-	-	*Anoxypristis cuspidata*	*A. cuspidata*	*A. cuspidata*	*A. cuspidata*
		Ic 3157	T120	-	-	-	*Anoxypristis cuspidata*	*A. cuspidata*	*A. cuspidata*	*A. cuspidata*
		Ic 3096	T121	-	-	-	*Anoxypristis cuspidata*	*A. cuspidata*	*A. cuspidata*	*A. cuspidata*
		Ic 3095	T122	-	-	-	*Anoxypristis cuspidata*	*A. cuspidata*	*A. cuspidata*	*A. cuspidata*
		Ic 2822	T123	Indian Ocean	-	-	*Anoxypristis cuspidata*	*A. cuspidata*	*A. cuspidata*	*A. cuspidata*
		Ic 3156	T124	-	-	-	*Anoxypristis cuspidata*	*A. cuspidata*	-	*A. cuspidata*
		Ic 3098	T125	-	-	-	*Anoxypristis cuspidata*	*A. cuspidata*	-	*A. cuspidata*
		Ic 3159	T126	-	-	-	*Anoxypristis cuspidata*	*A. cuspidata*	-	*A. cuspidata*
		Ic 2982	T127	-	-	-	*Anoxypristis cuspidata*	*A. cuspidata*	-	*A. cuspidata*
		Ic 3158	T128	-	-	-	*Anoxypristis cuspidata*	*A. cuspidata*	-	*A. cuspidata*
Natural history museum of Venice	27	20276	Ve147	-	-	-	*Pristis spp.*	*P. zijsron*	-	*P. zijsron*
		n.11292	Ve148	-	-	-	*Pristis spp.*	*P. pristis*	-	*P. pristis*
		n.11291	Ve149	-	-	-	*Pristis spp.*	*P. pristis*	-	*P. pristis*
		20272	Ve150	-	-	-	*Pristis spp.*	*P. zijsron*	-	*P. zijsron*
		19839/A	Ve151	-	-	-	*Pristis spp.*	*A. cuspidata*	-	*A. cuspidata*
			Ve152	-	-	-	*Pristis spp.*	*P. pectinata*	-	*P. pectinata*
		4723	Ve153	West Indian Ocean	African coasts	1898-1929	*Pristis spp.*	*P. zijsron*	-	*P. zijsron*
		20287	Ve154	-	-	-	*Pristis spp.*	*P. zijsron*	-	*P. zijsron*

		20280	Ve155	-	-	-	*Pristis spp.*	*P. zijsron*	-	*P. zijsron*
		20284	Ve156	-	-	-	*Pristis spp.*	*P. zijsron*	-	*P. zijsron*
		20274	Ve157	-	-	-	*Pristis spp.*	*P. zijsron*	-	*P. zijsron*
		20354	Ve158	-	-	-	*Pristis spp.*	*P. pristis*	-	*P. pristis*
		20353	Ve159	-	-	-	*Pristis spp.*	*P. pristis*	-	*P. pristis*
		20281	Ve160	-	-	-	*Pristis spp.*	*P. zijsron*	-	*P. zijsron*
		20282	Ve161	-	-	-	*Pristis spp.*	*P. zijsron*	-	*P. zijsron*
		20275	Ve162	-	-	-	*Pristis spp.*	*P. zijsron*	-	*P. zijsron*
		20277	Ve163	-	-	-	*Pristis spp.*	*P. zijsron*	-	*P. zijsron*
		20279	Ve164	-	-	-	*Pristis spp.*	*P. zijsron*	-	*P. zijsron*
		20283	Ve165	-	-	-	*Pristis spp.*	*P. zijsron*	-	*P. zijsron*
		20286	Ve166	-	-	-	*Pristis spp.*	*P. pristis*	-	*P. pristis*
		20278	Ve167	-	-	-	*Pristis spp.*	*P. pristis*	-	*P. pristis*
		20285	Ve168	-	-	-	*Pristis spp.*	*P. zijsron*	-	*P. zijsron*
		20273	Ve169	-	-	-	*Pristis spp.*	*P. zijsron*	-	*P. zijsron*
		19935	Ve170	-	-	-	*Pristis spp.*	*P. pectinata*	-	*P. pectinata*
		3527	Ve171	West Indian Ocean	Somalia, Adale	1962-1966	*Pristis microdon*	*P. pristis*	-	*P. pristis*
		3526	Ve172	West Indian Ocean	Somalia, Adale	1962-1966	*Pristis pectinata*	*P. zijsron*	-	*P. zijsron*
		3528	Ve173	West Indian Ocean	Somalia, Adale	1962-1966	*Pristis microdon*	*P. pristis*	-	*P. pristis*
Museum of Natural History "La Specola", University of Firenze	20	578	F1	-	-	-	*Pristis spp.*	*P. zijsron*	*P. zijsron*	*P. zijsron*
		573	F2	West Indian Ocean	Strait of Hormuz	1923	*Pristis pectinata*	*P. zijsron*	*P. zijsron*	*P. zijsron*
		6040	F3	-	-	-	*Pristis pectinata*	*P. zijsron*	*P. zijsron*	*P. zijsron*
		6357	F4	-	-	-	*Pristis spp.*	*P. zijsron*	*P. zijsron*	*P. zijsron*
		6358	F5	-	-	-	*Pristis spp.*	*P. pectinata*	*P. zijsron*	*P. zijsron*
		6360	F6	-	-	-	*Pristis spp.*	*P. zijsron*	*P. zijsron*	*P. zijsron*
		6112	F7	Mediterranean Sea	Messina, Tyrrhenian Sea	1837	*Pristis pectinata*	*P. pectinata*	*P. pectinata*	*P. pectinata*
		577	F8	-	-	-	*Pristis spp.*	*P. zijsron*	*P. zijsron*	*P. zijsron*
		6363	F9	-	-	-	*Pristis spp.*	*A. cuspidata*	*A. cuspidata*	*A. cuspidata*
		6344	F10	-	-	-	*Pristis spp.*	*A. cuspidata*	*A. cuspidata*	*A. cuspidata*

		6359	F11	-	-	-	*Pristis spp.*	*P. pristis*	*P. pristis*	*P. pristis*
		6081	F12	-	-	1959	*Pristis pristis*	*P. pristis*	*P. pristis*	*P. pristis*
		571	F13	-	-	-	*Pristis spp.*	*P. pectinata*	*P. zijsron*	*P. zijsron*
		583	F14	West Indian Ocean	Eritrea, Assab	1903	*Pristis spp.*	*P. zijsron*	*P. zijsron*	*P. zijsron*
		576	F15	-	-	-	*Pristis spp.*	*P. pectinata*	-	*P. pectinata*
		570	F16	-	-	1843	*Pristis spp.*	*P. pristis*	*P. pristis*	*P. pristis*
		575	F17	-	-	-	*Pristis spp.*	*P. zijsron*	*P. zijsron*	*P. zijsron*
		6362	F18	-	-	-	*Pristis spp.*	*P. zijsron*	*P. zijsron*	*P. zijsron*
		582	F19	-	-	-	*Pristis pectinata*	*P. pectinata*	*P. pectinata*	*P. pectinata*
		572	F20	-	-	-	*Pristis spp.*	*P. zijsron*	*P. zijsron*	*P. zijsron*
Wilderness studi ambientali (PA)	15	-	BZ89	-	-	-	*Pristis zijsron*	*P. zijsron*	*P. zijsron*	*P. zijsron*
		-	BZ90	Est Atlantic Ocean	Congo	-	*Pristis spp.*	*P. pectinata*	*P. pectinata*	*P. pectinata*
		-	BZ91	Est Atlantic Ocean	Congo	-	*Pristis pristis*	*P. pristis*	*P. pristis*	*P. pristis*
		-	BZ92	-	-	-	*Anoxypristis cuspidata*	*A. cuspidata*	*A. cuspidata*	*A. cuspidata*
		-	BZ93	West Pacific Ocean	-	-	*Anoxypristis cuspidata*	*A. cuspidata*	*A. cuspidata*	*A. cuspidata*
		-	BZ94	-	-	-	*Pristis spp.*	*P. pectinata*	*P. pectinata*	*P. pectinata*
		-	BZ95	-	-	-	*Pristis pectinata*	*P. pectinata*	*P. pectinata*	*P. pectinata*
		-	BZ96	-	-	-	*Pristis zijsron*	*P. zijsron*	*P. zijsron*	*P. zijsron*
		-	BZ97	West Indian Ocean	Madagascar	-	*Pristis pristis*	*P. pristis*	*P. pristis*	*P. pristis*
		-	BZ98	Est Atlantic Ocean	-	-	*Pristis pectinata*	*P. pectinata*	*P. pectinata*	*P. pectinata*
		-	BZ99	-	-	-	*Pristis zijsron*	*P. zijsron*	*P. zijsron*	*P. zijsron*
		-	BZ100	-	-	-	*Pristis zijsron*	*P. zijsron*	*P. zijsron*	*P. zijsron*
		-	BZ101	-	-	-	*Pristis spp.*	*P. pectinata*	*P. pectinata*	*P. pectinata*
		-	BZ102	-	-	-	*Pristis spp.*	*P. zijsron*	*P. zijsron*	*P. zijsron*
		-	BZ103	-	-	-	*Pristis pectinata*	*P. pectinata*	*P. pectinata*	*P. pectinata*
Natural history museum of Voghera (PV)	7	239	Vo129	-	-	-	*Pristis spp.*	*A. cuspidata*	*A. cuspidata*	*A. cuspidata*
		-	Vo130	-	-	-	*Pristis spp.*	*A. cuspidata*	*A. cuspidata*	*A. cuspidata*
		-	Vo131	-	-	-	*Pristis spp.*	*P. pristis*	*P. pristis*	*P. pristis*
		-	Vo132	-	-	-	*Pristis spp.*	*A. cuspidata*	*A. cuspidata*	*A. cuspidata*
		-	Vo133	-	-	-	*Pristis spp.*	*P. pectinata*	*P. pectinata*	*P. pectinata*

		-	Vo134	-	-	-	*Pristis spp.*	-	*P. pectinata*	*P. pectinata*
		2054	Vo135	-	-	-	*Pristis spp.*	*P. pristis*	*P. pristis*	*P. pristis*
Zoological Museum "P. Doderlein", University of Palermo	5	AN185	Pal106	-	-	<1895	*Pristis spp.*	*P. pectinata*	*P. pectinata*	*P. pectinata*
		AN186	Pal107	West Indian Ocean	Red Sea	-	*Pristis zijsron*	*P. zijsron*	*P. zijsron*	*P. zijsron*
		AN183	Pal108	West Indian Ocean	Red Sea	-	*Pristis zijsron*	*P. zijsron*	*P. zijsron*	*P. zijsron*
		AN182	Pal109	Mediterranean Sea	Palermo, Tyrrhenian Sea	-	*Anoxypristis cuspidata*	*A. cuspidata*	-	*A. cuspidata*
		AN184	Pal110	Mediterranean Sea	Palermo, Tyrrhenian Sea	-	*Pristis pectinata*	*P. pectinata*	-	*P. pectinata*
Zoological collection, University of Bologna	4	-	B136	-	-	-	*Pristis spp.*	*P. pristis*	-	*P. pristis*
		AC143	B137	-	-	-	*Pristis spp.*	*P. pristis*	-	*P. pristis*
		100138	B138	West Indian Ocean	Mozambique	-	*Pristis pectinata*	*P. zijsron*	-	*P. zijsron*
		2 (B25)	B139	Atlantic Ocean	-	1900	*Pristis pectinata*	*P. pristis*	*P. pristis*	*P. pristis*
Natural history museum of Dubrovnik	4	PMD 19	C73	-	-	-	*Pristis pectinata*	*P. zijsron*	*P. zijsron*	*P. zijsron*
		PMD 20	C74	Mediterranean Sea	Croatia, Dubrovnik	-	*Pristis pectinata*	*P. zijsron*	*P. zijsron*	*P. zijsron*
		PMD 21	C75	-	-	-	*Pristis pectinata*	*P. zijsron*	*P. zijsron*	*P. zijsron*
		PMD 22	C76	-	-	-	*Pristis pectinata*	*P. zijsron*	*P. zijsron*	*P. zijsron*
Natural history museum of Rovereto	4	P20	R64	Mediterranean Sea	Italy, Genova	1900	*Pristis spp.*	*P. zijsron*	*P. zijsron*	*P. zijsron*
		P21	R65	Indian Ocean	-	1939	*Pristis microdon*	*P. pristis*	*P. pristis*	*P. pristis*
		P18	R66	Mediterranean Sea	Venezia, Adriatic Sea	1900	*Anoxypristis cuspidata*	*A. cuspidata*	*A. cuspidata*	*A. cuspidata*
		P19	R67	Mediterranean Sea	Italy, Genova	1900	*Anoxypristis cuspidata*	*A. cuspidata*	*A. cuspidata*	*A. cuspidata*
Natural History Museum, University of Pisa	4	Pe 0116	Pi60	Mediterranean Sea	-	-	*Anoxypristis cuspidata*	*A. cuspidata*	*A. cuspidata*	*A. cuspidata*
		P171	Pi61	-	-	-	*Pristis spp.*	*P. zijsron*	*P. zijsron*	*P. zijsron*
		P172	Pi62	-	-	-	*Pristis spp.*	*P. zijsron*	*P. zijsron*	*P. zijsron*
		P488	Pi63	-	-	-	*Pristis spp.*	*P. zijsron*	*P. zijsron*	*P. zijsron*
Museum of comparative anatomy "Battista Grassi", Sapienza	3	-	RM86	West Indian Ocean	Red Sea	1939	*Pristis zijsron*	*P. zijsron*	*P. zijsron*	*P. zijsron*
		FA0003	RM87	-	-	1600	*Pristis spp.*	*P. pectinata*	*P. pectinata*	*P. pectinata*
		FA00035	RM88	-	-	-	*Pristis pristis*	*P. pectinata*	*P. pectinata*	*P. pectinata*
	3	107 127	N77	Mediterranean Sea	-	-	*Pristis pectinata*	*P. zijsron*	*P. zijsron*	*P. zijsron*

Museum of zoology, University of Navarra		107 129	N78	Mediterranean Sea	-	-	*Anoxypristis cuspidata*	*A. cuspidata*	*A. cuspidata*	*A. cuspidata*
		107 145	N79	-	-	-	*Pristis zijsron*	*P. zijsron*	*P. zijsron*	*P. zijsron*
Natural history museum of Verona	3	CO289	V80	West Indian Ocean	Somalia, Benadir	1935	*Pristis pristis*	*P. pristis*	*P. pristis*	*P. pristis*
		CO291	V81	West Indian Ocean	Tadjoura, Gibuti	1898	*Pristis zijsron*	*P. zijsron*	*P. zijsron*	*P. zijsron*
		CO292	V82	West Indian Ocean	Somalia, Benadir	1935	*Pristis pristis*	*P. pristis*	*P. pristis*	*P. pristis*
Private collection (C.P.)	3	-	Picci83	West Indian Ocean	Red Sea	1939	*Pristis zijsron*	*P. zijsron*	*P. zijsron*	*P. zijsron*
		-	Picci84	West Indian Ocean	Red Sea	1939	*Pristis zijsron*	*P. zijsron*	*P. zijsron*	*P. zijsron*
		-	Picci85	West Indian Ocean	Red Sea	1939	*Pristis zijsron*	*P. zijsron*	*P. zijsron*	*P. zijsron*
Comparative anatomy collection, University of Bologna	2	100944	B22	Mediterranean Sea	Adriatic Sea	-	*Pristis marsilii (?)*	-	*P. zijsron*	*P. zijsron*
		-	B24	-	-	1813	*Pristis spp.*	-	*P. pectinata*	*P. pectinata*
Croatian Museum of Natural History	2	417	C141	-	-	-	*Pristis pectinata*	*P. pectinata*	-	*P. pectinata*
		425	C142	-	-	-	*Pristis pectinata*	*A. cuspidata*	-	*A. cuspidata*
Museo Natura, Sant'Alberto	2	V281	S144	-	-	-	*Pristis pristis*	*P. pristis*	-	*P. pristis*
		V225	S145	-	-	-	*Pristis spp.*	*P. pectinata*	-	*P. pectinata*
Museum of Zoology, University of Padova	2	P29e	P58	Mediterranean Sea	Adriatic Sea	<1730	*Pristis pectinata*	*P. pectinata*	*P. pectinata*	*P. pectinata*
		P28e	P59	Mediterranean Sea	Adriatic Sea	<1730	*Pristis pectinata*	*P. zijsron*	*P. zijsron*	*P. zijsron*
National Museum of Zadar, Department of Natural History	2	ZDR26a	C70	-	-	-	*Pristis pectinata*	*P. zijsron*	*P. zijsron*	*P. zijsron*
		ZDR26b	C143	-	-	-	*Pristis pectinata*	*P. zijsron*	-	*P. zijsron*
Natural history museum of Rijeka	2	PMR 04432	C68	-	-	-	*Pristis pectinata*	*P. zijsron*	*P. zijsron*	*P. zijsron*
		PMR 04441	C69	-	-	-	*Pristis pectinata*	*P. zijsron*	*P. zijsron*	*P. zijsron*
Natural history museum of Split	2	PMST 10	C71	Mediterranean Sea	Southern Adriatic	1901	*Pristis pectinata*	*P. zijsron*	*P. zijsron*	*P. zijsron*
		PMST 11	C72	Indian Ocean	-	1929	*Pristis pectinata*	*P. zijsron*	*P. zijsron*	*P. zijsron*
	2	-	BZ104	Mediterranean Sea	-	-	*Pristis pristis*	*P. pristis*	*P. pristis*	*P. pristis*

Regional Museum of Terrasini		1134	BZ105	West Indian Ocean	Red Sea	-	*Pristis zijsron*	*P. zijsron*	*P. zijsron*	*P. zijsron*
Casa Matha, Ravenna	1	-	CM21	-	-	-	*Pristis spp.*	*P. zijsron*	*P. zijsron*	*P. zijsron*
Natural history museum of Comiso	1	-	Co140	-	-	-	*Pristis spp.*	*P. zijsron*	-	*P. zijsron*
Private collection (J)	1	-	J146	-	-	-	*Pristis spp.*	*A. cuspidata*	-	*A. cuspidata*
Private collection (P.B.)	1	-	Par57	-	-	-	*Pristis spp.*	*P. zijsron*	*P. zijsron*	*P. zijsron*
Private collection (I.M.)	1	-	IM174	-	-	<1955	*Pristis spp.*	*P. zijsron*	-	*P. zijsron*

Table S4 Details of linear rostra measurements, number of teeth per side and estimated TL of each sample.

Species morpho	COD_INV	ID_GEN	TSL	TRL	SRL	PRW	DRW	PTG	DTG	Nd	Ns	Estimated TL
A. cuspidata	6363	F9	775	730	605	63	40	28	9	28	26	3124.32
	6344	F10	668	668	523	53	33	31	8	24	26	2898.13
	Ic 3085	T38	445	404	322	40	21	16	4	27	29	2172.01
	Ic 3086	T40	850	750	644	61	32	25	8	30	29	3223.86
	Ic 3087	T41	825	717	555	59	41	25	7	27	26	2989.46
	Ic 3097	T43	560	560	478	50	25	25	5	27	27	2761.72
	Ic 3088	T44	610	546	414	42	22	26	5	28	25	2547.17
	Ic 3090	T45	510	510	410	44	25	18	5	26	24	2532.77
	Ic 3089	T46	630	630	490	48	33	26	7	26	24	2799.11
	Ic 3091	T47	570	565	443	46	31	23	5	25	27	2647.85
	Ic 3092	T48	485	443	330	43	21	18	4	27	27	2209.25
	Ic 3093	T49	685	685	500	57	31	23	7	26	26	2829.68
	Ic 2931	T52	625	625	527	52	25	30	5	28	27	2909.79
	Ic 2934	T55	690	670	585	58	31	32	8	28	28	3071.43
	Pe 0116	Pi60	-	370	271	28	15	13	4	24	24	1899.14
	P18 (N40)	R66	580	500	364	42	20	22	8	24	23	2356.19
	P19 (N41)	R67	700	580	445	43	24	21	4	26	25	2654.57
	107 129	N78	549	549	398	54	38	20	7	17	16	2488.72
	-	BZ92	620	620	505	60	45	30	8	23	22	2844.78
	-	BZ93	655	655	510	51	30	31	6	21	23	2859.75
	AN182	Pal109	416	416	339	40	20	22	5	23	25	2249.82
	Ic 3069	T117	280	245	200	20	15	12	4	32	32	1153.95
	Ic 3094	T118	854	844	690	65	38	32	6	29	29	3335.81

	Ic 3155	T119	755	710	565	58	33	31	5	28	27	3017.15
	Ic 3157	T120	685	685	605	57	38	26	9	26	27	3124.32
	Ic 3096	T121	616	616	490	54	27	28	7	26	25	2799.11
	Ic 3095	T122	720	600	465	45	34	24	10	24	26	2720.30
	Ic 3158	T123	754	730	557	56	31	29	11	26	25	2995.03
	Ic 3156	T124	728	728	560	58	30	30	7	27	26	3003.35
	Ic 3098	T125	478	478	340	38	22	22	4	25	24	2254.25
	Ic 3159	T126	720	720	495	50	34	24	8	24	25	2814.46
	Ic 2982	T127	1280	370	283	30	18	16	3	22	23	1970.25
	Ic 2822	T128	1270	335	239	30	15	18	3	22	23	1673.90
	239	Vo129	588	588	460	50	34	20	6	24	23	2704.11
	-	Vo130	690	690	556	57	35	29	7	27	28	2992.25
	-	Vo132	726	726	518	55	31	22	5	30	28	2883.46
	425	C142	1245	1245	1075	120	65	51	12	31	31	4119.21
	-	J146		450	390	45	25	20	5	26	25	2458.62
	19839/A	Ve151	340	338	270	30	17	15	4	28	28	1892.96
P. pectinata	6112	F7	1185	312	270	46	27	16	6	26	27	1261.26
	576	F15	398	232	200	33	17	15	4	24	24	937.86
	582	F19	1790	390	350	55	30	18	6	29	28	1576.58
	Ic 3106	T34	1040	933	870	134	55	55	15	26	26	3771.66
	P29e	P58	894	840	806	109	50.1	56.3	14	23	24	3395.71
	FA0003	RM87	860	860	816	140	61	45	20	21	22	3476.56
	FA00035	RM88	884	884	820	109	52	45	18	26	24	3573.58
	-	BZ90	875	840	803	115	51	56	16	23	22	3395.71
	-	BZ94	595	595	550	80	40	37	11	24	25	2405.29

	-	BZ95	600	575	530	85	40	29	12	26	27	2324.44
	-	BZ98	930	910	860	115	46	48	18	27	26	3678.68
	-	BZ101	630	630	615	100	50	25	10	24	25	2546.78
	-	BZ103	-	362	347	52	28	20	6	27	26	1463.39
	AN185	Pal106	1150	905	860	150	61	52	20	24	25	3658.47
	AN184	Pal110	274	274	200	37	20	19	5	23	24	1107.65
	-	Vo133	328	328	285	43	21	17	7	28	27	1325.94
	417	C141	225	225	204	33	20	11	6	25	24	909.56
	V225	S145	470	470	442	80	38	24	10	20	22	1899.98
	-	Ve152	940	940	895	120	65	55	17			3799.96
	19935	Ve170		218	200	30	19	12	5	30	31	881.27
P. pristis	6359	F11	755	755	717	142	58	32	27	20	20	3567.39
	6081	F12	1052	875	850	174	65	47	33	21	20	4333.29
	570	F16	1200	1130	1120	205	78	58	45	21	20	5939.45
	Ic 3099	T26	1590	1350	1200	237	82	68	45	20	19	6426.79
	Ic 3101	T27	1070	1070	980	213	78	52	34	18	17	5098.74
	Ic 3100	T33	770	770	700	145	51	43	23	20	20	3470.88
	Ic 3102	T37	1100	1100	1070	218	91	55	50	17	17	5637.37
	Ic 2826	T50	1620	1365	1265	280	105	80	47	17	17	6826.20
	Ic 2827	T51	1240	1075	1038	234	91	69	45	18	18	5445.08
	P21 (N43)	R65	1230	1160	1090	203	85	63	35	18	18	5757.96
	CO289	V80	777	750	717	142	56	42	30	18	20	3567.39
	CO292	V82	779	755	717	142	58	44	27	17	18	3567.39
	-	BZ91	770	770	658	128	52	49	30	22	21	3233.89
	-	BZ97	854	854	790	156	60	42	21	21	19	3985.47

	-	BZ104	1150	1150	1060	241	76	67	46	24	24	5577.19
	Ic 3160	T112	547	547	535	120	52	25	25	16	18	2552.72
	-	Vo131	647	647	595	128	47	30	25	18	17	2882.48
	2054	Vo135	1075	1075	980	208	78	60	45	15	15	5098.74
	-	B136	787	787	716	152	53	40	26	20	21	3561.71
	AC143	B137	602	582	546	99	43	35	21	21	19	2612.80
	-	B139	225	197	189	32	15	11	6	24	24	777.13
	V281	S144	1180	1180	1140	245	82	62	41	19	19	6060.84
	n.11292	Ve148	1322	1322	1240	245	100	78	47	18	19	6672.23
	n.11291	Ve149	1190	1190	1105	235	115	60	38	17	17	5848.62
	20354	Ve158	1105	1105	1010	200	90	53	33	20	19	5277.52
	20353	Ve159	1115	1115	990	205	92	59	38	21	18	5158.25
	20286	Ve166	980	880	830	160	74	55	34	16	18	4216.95
	20278	Ve167	880	880	805	159	72	48	30	18	18	4072.08
	3527	Ve171	1105	1105	1090	235	105	60	43	18	19	5757.96
	3528	Ve173	850	850	790	157	71	42	25	20	19	3985.47
P. zijsron	578	F1	293	290	263	38	20	23	4	27	26	1103.46
	573	F2	1252	1250	1110	128	52	63	12	33	35	3784.74
	6040	F3	1400	1340	1200	132	58	70	17	30	31	4045.91
	6357	F4	275	260	222	31	18	20	3	26	26	954.46
	6358	F5	186	186	171	29	15	10	5	26	27	763.36
	6360	F6	950	900	830	110	42	53	14	28	28	2951.05
	577	F8	298	298	265	36	19	18	4	29	29	1110.64
	571	F13	448	448	400	63	27	27	8	23	23	1579.89
	583	F14	1345	380	338	45	22	18	5	29	28	1367.79

575	F17	280	267	236	31	19	19	5	23	23	1005.75
6362	F18	1285	1285	1119	138	55	77	16	32	32	3810.99
572	F20	1650	1450	1270	130	58	118	12	30	30	4247.09
-	CM21	1260	1260	1234	126	55	87	11	29	29	4143.83
Ic 3105	T28	1020	970	860	112	48	53	12	33	32	3042.12
Ic 3113	T29	1500	1390	1240	135	56	80	18	30	31	4161.07
Ic 3108	T30	1280	1280	1145	125	53	65	15	31	32	3886.65
Ic 3110	T31	1390	1319	1170	145	65	85	15	31	30	3959.18
Ic 3111	T32	1290	1174	1120	125	57	75	12	29	29	3813.90
Ic 3109	T35	1330	1215	1160	145	55	75	9	32	35	3930.20
Ic 3107	T36	1200	1141	985	120	49	57	13	30	29	3416.83
Ic 3104	T39	700	625	578	74	37	35	9	33	33	2165.04
Ic 3112	T42	1380	1380	1240	132	54	82	12	34	33	4161.07
Ic 2932	T53	990	930	874	120	49	63	10	30	28	3084.46
Ic 2933	T54	1240	1144	1060	125	57	51	13	31	31	3638.33
Ic 2935	T56	1265	1150	1087	116	51	65	12	30	30	3717.51
-	Par57	520	518	484	59	29	27	6	32	32	1859.89
P28e	P59	1089	1089	1003	114	49.8	43.8	10	32	32	3470.21
P171	Pi61	1020	1020	972	102	47	68	9	30	30	3378.19
P172	Pi62	930	930	798	89	43	57	10	28	27	2853.39
P488	Pi63	1180	1100	975	103	53	70	11	29	28	3387.12
P20 (N42)	R64	640	500	461	52	30	34	6	32	33	1783.97
PMR 04432	C68	-	1320	1163	135	67	94	13	32	30	3938.89

PMR 04441	C69	1289	1289	1112	135	73	92	12	33	32	3790.57
ZDR26a	C70	840	724	653	98	65	52	6			2403.35
PMST 10	C71	743	677	659	80	37	40	9	32	31	2422.24
PMST 11	C72	1045	1045	1006	122	53	79	11	29	32	3479.09
PMD 19	C73	1476	1318	1265	148	62	70	8	33	34	4232.78
PMD 20	C74	1376	1270	1215	139	53	66	10	32	32	4089.16
PMD 21	C75	1264	1197	1140	124	58	93	12	31	30	3872.12
PMD 22	C76	1168	1100	1040	124	60	65	13	29	29	3579.49
107 127	N77	692	638	572	65	36	31	8	30	31	2145.78
107 145	N79	1236	1236	1156	118	62	88	15	27	27	3918.59
CO291	V81	809	771	692	80	43	39	8	32	31	2525.70
-	Picci83	853	744	660	92	47	48	10	29	28	2425.39
-	Picci84	830	719	623	76	37	44	10	30	27	2308.53
-	Picci85	820	592	528	73	32	30	8	31	30	2003.70
-	RM86	1554	1373	1241	130	74	96	18	30	29	4163.95
-	BZ89	1470	1470	1270	136	60	110	10	25	25	4247.09
-	BZ96	650	600	562	70	30	28	5	36	35	2113.63
-	BZ99	1110	1110	964	114	47	67	10	28	29	3354.38
-	BZ100	1110	1080	980	115	42	92	17	33	35	3401.98
-	BZ102	890	300	262	35	25	17	5	24	24	1099.87
1134	BZ105	1220	1220	1160	128	52	83	10	31	30	3930.20
AN186	Pal107	1330	1330	1174	135	56	100	16	31	30	3970.76
AN183	Pal108	1210	1160	1030	111	50	89	9	29	29	3550.01
Ic 3114	T111	1270	1270	1165	135	60	75	13	30	31	3944.69

Ic 3161	T113	995	920	795	108	58	65	17	22	23	2844.21
Ic 3103	T114	207	207	200	29	15	12	3	30	31	872.90
Ic 3070	T115	242	200	180	23	15	12	4	28	29	797.62
Ic 3162	T116	200	165	153	23	15	8	1.5	26	25	694.04
100138	B138	990	980	840	109	54	53	12	31	32	2981.46
-	Co140	780	583	568	74	46	36	7	30	30	2132.93
ZDR26b	C143	1490	1372	1246	123	58	94	13	30	30	4178.30
20276	Ve147	1375	1335	1210	137	72	77	16	30	30	4074.75
20272	Ve150	1138	1107	997	113	64	74	14	27	29	3452.43
4723	Ve153	1300	1220	1125	118	58	68	9	32	32	3828.47
20287	Ve154	1193	1193	1150	130	80	70	14	26	26	3901.18
20280	Ve155	1290	1290	1200	130	70	104	11	30	30	4045.91
20284	Ve156	1370	1275	1185	135	65	65	12	33	33	4002.58
20274	Ve157	1325	1215	1135	125	62	75	13	28	28	3857.58
20281	Ve160	1395	1250	1165	130	70	70	12	32	31	3944.69
20282	Ve161	1425	1315	1190	133	72	85	14	30	30	4017.04
20275	Ve162	1330	1240	1170	128	67	90	14	29	29	3959.18
20277	Ve163	1365	1240	1112	126	60	85	11	29	27	3790.57
20279	Ve164	1415	1280	1180	132	67	95	12	29	32	3988.12
20283	Ve165	1410	1260	1186	130	75	63	13	28	29	4005.48
20285	Ve168	873	820	763	89	51	38	8	32	30	2745.93
20273	Ve169	985	975	893	95	54	64	9	32	32	3141.76
3526	Ve172	1027	970	898	105	55	64	7	30	30	3156.81
-	IM174	1440	1340	1240	143	65	85	16	33	32	4161.07

Chapter 3

Temperature tolerance and geographical origin of sawfishes through measurements of stable isotopes of Oxygen and Carbon of historical rostral teeth

3.1 Introduction

In the last decades, stable isotope analysis has emerged as a powerful tool in ecological research with wide application in the trophic ecology, habitat use, movement, and migration patterns (Hobson, 1999; Layman et al., 2012; Ramos & González-Solís, 2012), source sharing and parasite-host interaction (Boecklen et al., 2011), and to infer past environmental changes in marine environments (Bigg & Rohling, 2000; Sisma-Ventura et al., 2019; Willmes et al., 2019). Stable isotope analysis involves the use of mass spectroscopy to determine the constituent forms of carbon, nitrogen, and other elements that make up the tissues of organisms (Reum, Williams, & Harvey, 2017). Thus, the recent ecology of the organism is directly and predictably reflected in the stable isotope ratios (Peterson & Fry, 1987) useful for many biological, ecological, archaeological, and other scientific investigations.

The constituent forms of carbon and nitrogen are the most common isotopes used to evaluate trophic food web or trophic chain (Rubenstein & Hobson, 2004; Logan & Lutcavage, 2010; Reum, Williams, & Harvey, 2017) and to track migration patterns of different marine species (Carlisle et al., 2012; Trueman, Mackenzie, & Palmer, 2012; Christiansen et al., 2014). In addition, oxygen isotopes are widely used to evaluate the isotopic composition of oceanic water, varying according to salinity

and depth (Bigg & Rohling, 2000; Willmes et al., 2019). For this reason, oxygen is considered a good proxy to estimate paleoclimatic conditions and differences from different water masses (Bigg & Rohling, 2000; Trueman, Mackenzie, & Palmer, 2012; Séon et al., 2020). Moreover, isotopes of oxygen and carbon could be used to build oceanic isoscapes (Torniainen et al., 2017a; Bird et al., 2018; Pearson, van de Merwe, & Connolly, 2020), offering many potential applications in environmental and ecological sciences. Extending isoscapes at the global scale allows assigning the probability of the distribution area to organisms with unknown origin (Bird et al., 2018).

Calcium carbonate hard structures, such as otoliths and teeth, trap similar elements and isotopes in their chemical matrix and are widely used for this kind of analysis (Vennemann et al., 2001; Field et al., 2009; Sisma-Ventura et al., 2019; Willmes et al., 2019). Focusing on elasmobranchs, stable isotope studies have yielded insight into a broad range of issues ranging from seasonal foraging and migration patterns to community functional diversity, intrapopulation resource partitioning and trophic position (e.g., MacNeil, Skomal, & Fisk, 2005; Matich, Heithaus, & Layman, 2010; Abrantes & Barnett, 2011; Borrell et al., 2011; Hussey et al., 2014).

Sawfishes (family Pristidae) are among the elasmobranch groups most affected by human threats (Dulvy et al., 2014; Yan et al., 2021) causing their dramatic reduction in many coastal areas worldwide. Rostral teeth of sawfishes are composed of a complex matrix of hydroxyapatite (Hegg, Graves, & Fisher, 2021) which can incorporate the oxygen and trace elements present in the environment (Combes, Cazalbou, & Rey, 2016). Carbonate fraction commonly substitutes the phosphate in the hydroxyapatite structure (Amiot et al., 2010; Crowley & Wheatley, 2014) thus, different compositions of isotopes can effectively discriminate among habitats and water masses (Field et al. 2009; Hegg, Graves, & Fisher, 2021). Sawfishes inhabit tropical-subtropical waters and are bentho-pelagic, spending their life in different habitat ranges such as freshwater (river or lake), estuarine, inshore regions and offshore waters to a maximum of 122 m in depth (Poulakis & Seitz, 2004; Wueringer, Squire, & Collin, 2009). The Mediterranean Sea has peculiar characteristics, and it is one of the historical areas in which sawfish are considered locally extinct (Ferretti et al., 2016). Their presence in the basin has been under debate for decades, thus considering sawfish as non-resident and occasional visitors (Faria et al., 2013; Dulvy et al., 2014). Nevertheless, bibliographic records and museum finds supported the Mediterranean Sea as formerly part of the Sawfish's distribution range. Ferretti et al. (2016) demonstrated the presence of two species in the basin, the Largetooth sawfish (*Pristis pristis* L.) and the Smalltooth sawfish (*P. pectinata* Latham, 1794) and estimated the period of local extinction during the past century.

This thesis chapter address geographical origin, past distribution and ecology of Mediterranean and global sawfish by 1) the assessment of isotopic composition analysis of oxygen ($^{18}O/^{16}O$) and carbon ($^{13}C/^{12}C$) in historical rostral teeth of specifically-identified specimens belonging to museum and private collections and 2) the comparison of individual isotope composition against the available environmental oceanic isoscapes.

3.2 Materials and methods

3.2.1 Sampling and preparation of rostral teeth

In total, 62 teeth were collected from the 172 museum rostra inventoried in the museum and private collections in the Mediterranean area (see Chapter 1, Table S3). Among them, only 35 teeth were used to assess their oxygen and carbon isotope composition, as the other 27 teeth were too damaged for a proper analysis. Each sample was prepared following the procedure described in Ventresca Miller et al. (2018).

Before pulverizing the teeth, the working area was covered with a thin layer of tinfoil. To avoid cross-contamination, the tinfoil was cleaned between one tooth and another using a paper wet of sodium hypochlorite (NaClO). The teeth were pulverized using a Dremel 4000 and four different diamond tips to collect the dental powder; the first diamond tip was used to polish the teeth from the external exposed layer, the second and the third tips were used in the same way to pulverise the teeth, and the last one was used to collect all the powder into 1.5 mL microtubes. Each tip was cleaned with NaClO after each sample.

To cover the whole animal life, at least 2.5 mg of tooth powder was collected from three different parts of the tooth crown, apex, middle and cervix. For 16 teeth (10-2.5 cm in length size), powder samples were collected from each of the three different parts, for six fragmented teeth (3.5-1.4 cm in length size) only two samples per tooth were collected, one from the middle and one from the apex or cervix, depending on the missing part; for the remaining 15 teeth (1.4-1.0 cm in length size), it was possible to collect only one sample representing the whole tooth length.

3.2.2 Oxygen and carbon isotope analysis

The oxygen and carbon isotope analyses of the dental carbonate of samples were conducted at the Department of Chemical and Geological Sciences of the University of Modena and Reggio-Emilia. Each sample (ca. 2 mg of dental powder) was reacted in glass vials with 100% H_3PO_4 at 70°C (Vennemann et al., 2001; Sisma-Ventura et al., 2019). The $\delta^{18}O$ and $\delta^{13}C$ values of the tooth powder samples were then determined on the liberated CO_2 using an Elementar Isoprime precision mass spectrometer, coupled with an Isoflow equilibrator. Analyses were run in collaboration with the Department of Physics and Earth Sciences of the University of Ferrara.

Raw data were corrected using an in-house marble reference material. The analytical precision of this method is better than ±0.1‰ for both $\delta^{18}O$ and $\delta^{13}C$. Measured isotope ratios are reported in δ-notation, i.e., as the deviation in per mil (‰) from the international measurement standards Vienna Standard Mean Ocean Water (VSMOW; $\delta^{18}O$) and Vienna Pee Dee Belemnite (VPDB; $\delta^{13}C$), and were calculated with the following formula:

$$\delta sample = \left[\frac{Rsample}{Rstandard} - 1\right] x10^3 \quad (1)$$

where Rsample and Rstandard represent the $^{18}O/^{16}O$ or $^{13}C/^{12}C$ ratio of the sample and of the standard, respectively (Sisma-Ventura et al., 2019). The ratios were obtained in $\delta^{13}C$VPDB and $\delta^{18}O_C$VPDB - where $\delta 18O_C$ corresponded to the oxygen isotopic composition of Carbonate in sawfish teeth. We calculated the density plot of $\delta^{13}C$VPDB and $\delta^{18}O_C$VPDB to evaluate the distribution of the data obtained. Data were compared with a reference dataset (Table S1) created using the available public data on the main subdivisions in different macro-groups of marine animals. The boxplots were carried out for $\delta^{13}C$VPDB and $\delta^{18}O_C$VPDB using different taxa and geographical locations.

Subsequently, $\delta^{18}O_c$ values were converted in VSMOW using the following relation:

$$\delta^{18}O_cVSMOW = (\delta^{18}O_c\text{VPDB}\ x\ 1.03086) + 30.86 \quad (2)$$

Moreover, each value of $\delta 18O_C$VSMOW were transformed in $\delta 18O_P$VMSOW using the relation between these values (Vennemann et al., 2001):

$$\delta^{18}O_p\text{VSMOW} = \delta^{18}O_cVSMOW + 9.1 \quad (3)$$

where $\delta^{18}O_p$ is the oxygen isotopic composition of Phosphate in sawfish teeth. Subsequently, $\delta^{18}O_C$VPDB and $\delta^{18}O_P$VSMOW were successively used to calculate the temperature for different sea using the formula (Lécuyer et al., 2013):

$$T° = 16 - 4.14(\delta^{18}O_cVPDB - \delta^{18}O_w\text{VSMOW}) + 0.13(\delta^{18}OCVPDB - \delta^{18}O_w\text{VSMOW})^2 \quad (4)$$

and (Vennemann et al., 2001):

$$T° = 111.4 - 4.3(\delta^{18}O_p\text{VSMOW} - \delta^{18}O_w\text{VSMOW}) \quad (5)$$

where each $\delta^{18}O_W$VSMOW were calculated using the bootstrap from all the values of $\delta^{18}O_W$ downloaded from https://data.giss.nasa.gov/o18data/ (Schmidt, 1999), using the coordinate of Indian Ocean (-0.614), Central Atlantic Ocean (0.630), Mediterranean Sea (1.464) and Red Sea (1.715).

Furthermore, to calculate the $\delta^{18}O_W$VMSOW for each sample and determine the area of origin, three possible temperatures at which sawfish usually inhabit have been chosen. For the lowest temperature were used 18°C, 25°C for the mean temperature, and the maxim temperature 32°C (Ferretti et al., 2016; Hollensead et al., 2016, 2018). The formula used is the opposite of the formula used above (5):

$$\delta^{18}O_w\text{VSMOW} = -25.9 + \delta^{18}O_p\text{VSMOW} + \left(\frac{T^\circ}{4,3}\right) \quad (6)$$

The estimated $\delta^{18}O$ of each tooth was compared against the corresponding distribution maps of seawater isotopes (i.e., isoscapes) to geographically assign the origin of museum and private samples. $\delta^{18}O$ isoscapes for global oceans were downloaded from https://data.giss.nasa.gov/o18data/ (Schmidt, 1999) for water depth between 0 and 50 m. These isoscapes were stacked in QGIS v. 3.18 and averaged using the raster calculator. However, the tissue/seawater isotopic fraction should be carefully considered to use these maps as predictors for the geographical origin of a sample. In particular, the isotopic composition of carbonate ($\delta^{18}O_c$) in fish skeletal tissues is mainly governed by the isotopic composition ($\delta^{18}O_w$) and the temperature (T) of the seawater. For this work, we used the equation for biogenic aragonite from Grossman and Ku (1986), to convert the $\delta^{18}O_w$ in $\delta^{18}O_c$:

$$\delta^{18}O_c = \frac{20.6 - 4.3 * \delta^{18}O_w - T}{4.3} \quad (7)$$

The temperature was determined from seawater surface temperature (STT) maps downloaded from oceandata.sci.gsfc.nasa.gov/directaccess/MODIS-Aqua/Mapped/Annual/4km/sst/ for 2020. Monthly maps were averaged in QGIS to obtain an annual mean SST map. Both the $\delta^{18}O_w$ isoscape and the mean annual SST map were imported as raster files in R version 4.1.0 (R Core Team, 2021). Being at a different resolution, the $\delta^{18}O_w$ isoscape was resampled at the same resolution of the SST map using the *resample* function of the '*raster*' package version 3.5-11 (Hijmans et al., 2021). The $\delta^{18}O_c$ isoscape was then calculated following the Grossman & Ku (1986) equation. To perform a probabilistic assignment of the sawfish specimens (based on their $\delta^{18}O_c$ data) and the obtained $\delta^{18}O_c$ ocean isoscape, we employed the R package '*assignR*' (Ma et al., 2020). In brief, with this package, it was possible to generate posterior probability density maps (considering 0.3 for the standard error), expressing how likely a specific sample originated from different parts of the input isoscape. The summed probability (i.e., all cell values) of these maps is equal to one. Each sample map was calculated using 0.1 standard error.

3.3 Results

The 35 rostral teeth suitable for the isotopic analysis belonged to *Pristis zijsron* Bleeker, 1851 (N = 25), *P. pristis* (N = 5), *P. pectinata* (N = 3) and *Anoxypristis cuspidata* Latham, 1794 (N = 2). Oxygen and carbon isotope compositions of apatite carbonate ($\delta^{18}O_C$VDPB and δ^{13}CVPDB respectively), as well as the derived values of $\delta 18O_C$VMSOW and the $\delta 18O_P$VMSOW, were listed in Table 1. $\delta^{18}O_C$VDPB values ranged from -4.48‰ (F19, *P. pectinata*) to -0.98‰ (T35, *P. zijsron*), while values of δ^{13}CVPDB ranged from -4.75‰ (F16, *P. pristis*) to 3.56‰ (F14, *P. zijsron*) (Table 1).

Table 1 Carbon and Oxygen composition of carbonate and phosphate from the 35 sawfish teeth.

Sample code	Species	Documental origin	Weight (mg)	δ^{13}C VPDB	$\delta^{18}O_C$ VPDB	$\delta^{18}O_C$ SMOW	$\delta d^{18}O_P$ SMOW
F2	*P. zijsron*	Indian Ocean, Strait of Hormuz	2.56	-0.27	-1.82	28.98	19.88
F14	*P. zijsron*	Red Sea, Assab, Eritrea	3.26	3.56	-3.18	27.58	18.48
T26	*P. pristis*	Indian Ocean, Madras, India	2.88	1.51	-3.15	27.62	18.52
P59	*P. zijsron*	Mediterranean Sea, Adriatic Sea	2.69	-1.72	-3.25	27.50	18.40
C71	*P. zijsron*	Mediterranean Sea Adriatic Sea	2.45	-0.13	-2.63	28.15	19.05
C72	*P. zijsron*	Indian Ocean	3.08	0.66	-3.27	27.49	18.39
C74	*P. zijsron*	Mediterranean Sea, Adriatic Sea	3.23	-1.16	-3.14	27.62	18.52
Picci84	*P. zijsron*	Red Sea	3.33	1.61	-2.81	27.96	18.86
F3	*P. zijsron*	-	2.83	-0.74	-2.14	28.65	19.55
F6	*P. zijsron*	-	2.34	0.78	-2.01	28.78	19.68
F9	*A. cuspidata*	-	2.92	-0.04	-2.38	28.41	19.31
F10	*A. cuspidata*	-	3.00	-0.11	-2.32	28.47	19.37
F11	*P. pristis*	-	2.17	0.50	-3.30	27.46	18.36
F12	*P. pristis*	-	3.30	-0.41	-3.35	27.40	18.30
F15	*P. pectinata*	-	2.14	-2.60	-2.51	28.27	19.17
F16	*P. pristis*	-	2.20	-4.75	-3.87	26.87	17.77
F17	*P. zijsron*	-	2.76	-2.84	-3.86	26.88	17.78
F18	*P. zijsron*	-	2.55	-1.29	-2.39	28.40	19.30
F19	*P. pectinata*	-	3.04	1.68	-4.48	26.24	17.14

F20	*P. zijsron*	-	2.45	-1.14	-3.55	27.21	18.11
T28	*P. zijsron*	-	3.33	-0.73	-1.77	29.03	19.93
T29	*P. zijsron*	-	3.39	0.32	-1.79	29.01	19.91
T30	*P. zijsron*	-	3.53	-0.16	-2.29	28.50	19.40
T31	*P. zijsron*	-	3.28	0.09	-1.61	29.20	20.10
T32	*P. zijsron*	-	2.64	-2.56	-3.43	27.32	18.22
T34	*P. pectinata*	-	2.81	-1.85	-2.56	28.22	19.12
T35	*P. zijsron*	-	3.01	-1.23	-0.98	29.85	20.75
T36	*P. zijsron*	-	3.61	-0.24	-1.61	29.20	20.10
T37	*P. pristis*	-	3.79	0.22	-3.24	27.52	18.42
Pi63	*P. zijsron*	-	3.75	-1.77	-2.31	28.48	19.38
C69	*P. zijsron*	-	2.72	-0.56	-2.62	28.16	19.06
C70	*P. zijsron*	-	1.00	0.15	-2.92	27.85	18.75
C73	*P. zijsron*	-	2.21	-2.72	-3.18	27.58	18.48
C75	*P. zijsron*	-	3.06	0.74	-2.10	28.70	19.60
C76	*P. zijsron*	-	3.30	-2.06	-2.98	27.79	18.69

The comparison of historical sawfish values with the available public data for the ancient extinct sawfish (*Onchopristis numidus*) of the middle Cretaceous, molluscs with Bivalvia and Cephalopoda, fish (fossil records of ancient extinct species and modern *Sparus aurata*) and sharks (from different modern species of Lamniformes, Carcharhiniformes and Squaliformes as well as fossil records of ancient extinct species) (see Table S1 and literature therein) showed an intermediate distribution between sharks and fish, meaning that they were similar in composition of the oxygen and carbon isotopic estimates (Figure 1). Our values were highly different from those obtained for the ancient euryhaline sawfish *O. numidus*, although the species may have shared the same living environment between fresh and shallow marine waters, reflecting the different environmental conditions of the middle Cretaceous. Moreover, similarities were observed in samples from the Central Atlantic (Atlantic C), Indian Ocean and the Red Sea (Figure 2), suggesting these areas among the geographical origin of our sawfish samples.

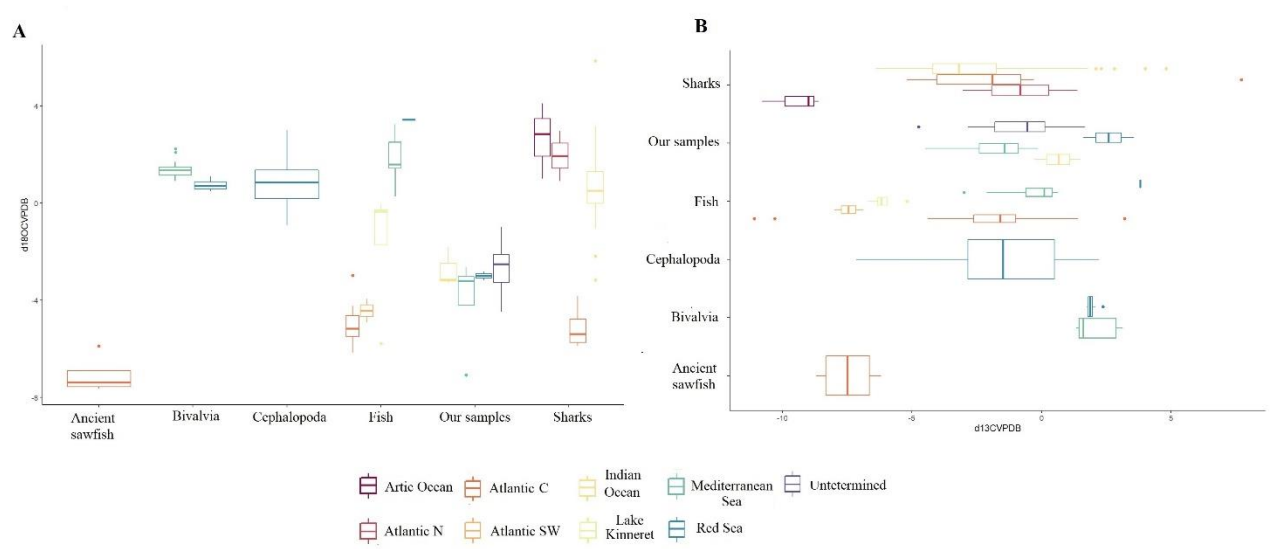

Figure 1 $\delta^{18}O_C$VPDB (A) and δ^{13}CVPDB (B) composition conducted on the reference database compared with our data. Values are plotted according to taxa and coloured according to geographical origin.

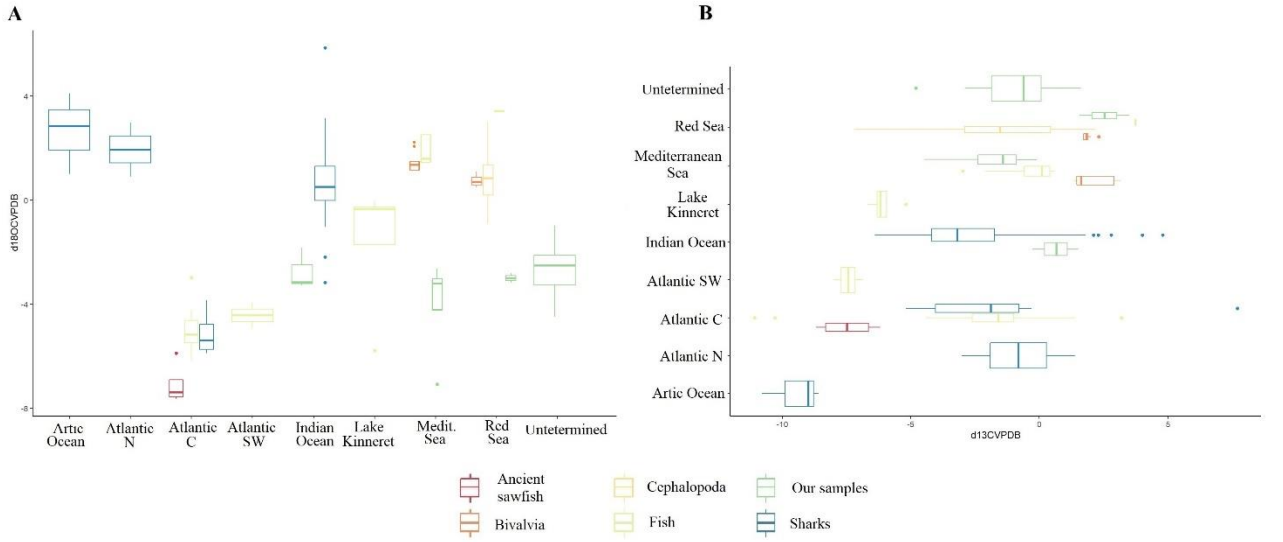

Figure 2 δ18O_CVPDB (A) and δ13CVPDB (B) composition conducted on the reference database compared with our data. Values are plotted according to geographical origin and coloured according to taxa.

The estimate of the possible sea temperature of the habitat of origin showed similar values between those calculated using $\delta^{18}O$, carbonate ($\delta^{18}O_C$) and phosphate ($\delta^{18}O_P$) (Table S2). Remarkable differences were obtained among seas (Figure 3). Temperatures estimated using $\delta^{18}O_W$VMSOW from the bootstrap value of the Indian Ocean appeared the most similar to the temperature range of the typical sawfish habitat (18-32°C), with the lowest temperature obtained for the sample T35 (19.54°C with $\delta^{18}O_P$ and 18.52°C with $\delta^{18}O_C$) and the higher for the sample F19 (35.06°C with $\delta^{18}O_P$ and 35.78°C with $\delta^{18}O_C$). Considering the bootstrap values of the Red Sea and the Mediterranean Sea we obtained higher values of temperatures, ranging from 29.55°C to 45.07°C ($\delta^{18}O_P$) and from 29.65°C to 49.02°C ($\delta^{18}O_C$) for the Red Sea, while for the Mediterranean Sea the range was from 28.47°C to 44.00°C ($\delta^{18}O_P$) and 28.38°C to 47.53°C ($\delta^{18}O_C$). When taking into account the Central Atlantic Ocean bootstrap value, the temperatures we obtained here were mostly high, ranging from 24.88°C to 40.41°C using $\delta^{18}O_P$ and from 24.29°C to 42.68°C using $\delta^{18}O_C$.

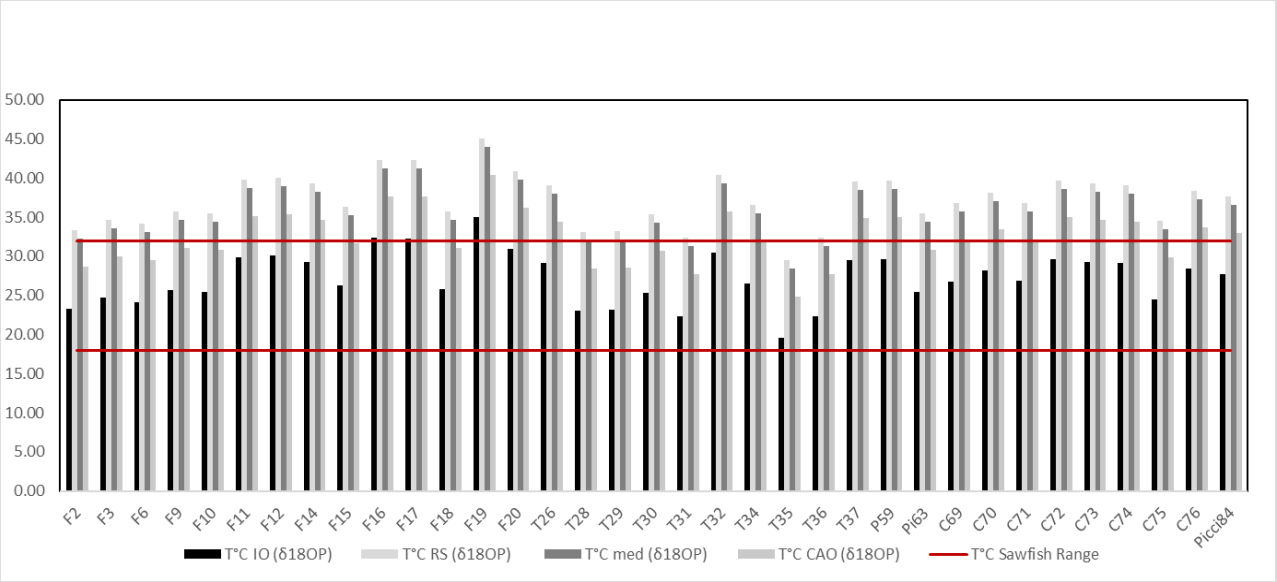

Figure 3 Results of estimated temperature using oxygen isotopes from phosphate for different seas using the coordinate of Indian Ocean (IO), Red Sea (RS), Mediterranean Sea (MED) and Central Atlantic Ocean (CAO). Red lines delimitate the sawfish temperature tolerance (18 – 32 °C).

The possible $\delta^{18}O_W$VMSOW calculated for the three temperatures (Table S2) showed how the three different temperatures were placed near $\delta^{18}O_W$VMSOW from the Indian Ocean, and only a few exceptions were present for the other areas.

A remarkable variation of the isotopic geographical origin was obtained among samples (Figure S1) with different cases of consistency/inconsistency with the related documental origin and cases of uncertain assignment (i.e., to multiple isoscapes). Only three individuals (F16, F17 and F19) without documental origin, showed low probabilities to be assigned.

Among the eight individuals with documental geographic origin (Table 1), only the specimen F14 (Figure 4) and probably the C72, both belonging to *P. zijsron*, showed a consistency between the isotopic origin from the Red Sea and/or the Persian Gulf (Figure 4 and S1) and the documental geographic origin from the museum legacy (F14: "Assab, Eritrea, Red Sea" C72: a generic "Indian Ocean"). Similarly, the *P. zijsron* Picci84, labelled from the Red Sea showed an isotopic origin from the Red Sea or Central-Western Atlantic Ocean (Figure 5). The *P. zijsron* individuals F2, labelled as Strait of Hormutz - Indian Ocean, and C71, labelled as Adriatic Sea - Mediterranean Sea, showed a high probability to originate from more areas such as the Indian Ocean, Pacific Ocean, Atlantic Ocean, and the Mediterranean Sea (Figure 6). On the contrary, the *P. pristis* T26 and the *P. zijsron* P59 and C74, showed an isotopic origin from the Red Sea and/or the Persian Gulf (Figure 7), fully inconsistent with the museum documental origin from Madras, India (T26) and Adriatic Sea (P59, C74).

Overall, three individuals out of eight showed a correspondence between documental and isotopic origin while three individuals not. Two individuals showed a documental origin coherent with at least one of the multiple origins obtained with isotope analysis.

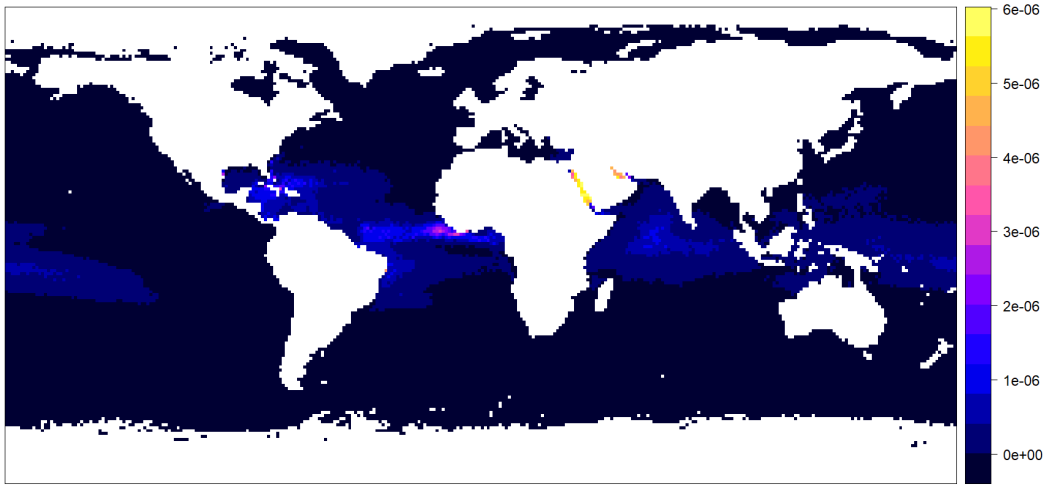

Figure 4 Estimated probability origin of the tooth sample F14 (documental geographic origin: "Assab, Eritrea, Red Sea") calculated from the values of the oxygen isotopes. Yellow colours showed the highest probability and dark blue the lowest.

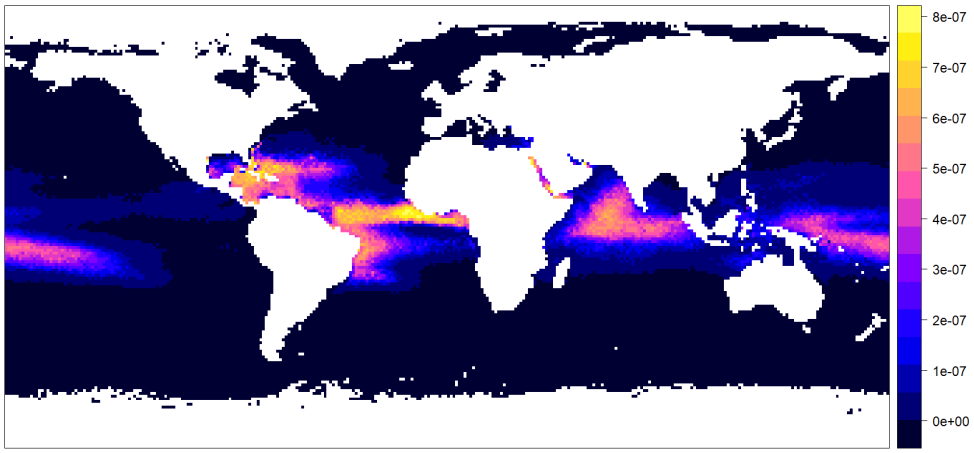

Figure 5 Estimated probability origin of the tooth sample Picci84 (attributed to the species *P. zijsron* and with documental geographic origin: "Red Sea") calculated from the values of the oxygen isotopes. Yellow colours showed the highest probability and dark blue the lowest.

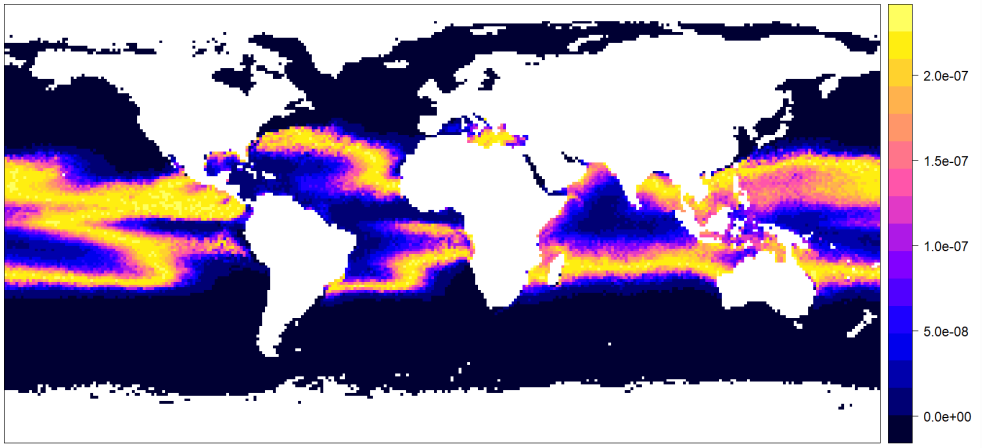

Figure 6 Estimated probability origin of the tooth sample F2 (attributed to the species *P. zijsron* and with documental geographic origin: "Strait of Hormutz, Indian Ocean") calculated from the values of the oxygen isotopes. Yellow colours showed the highest probability and dark blue the lowest.

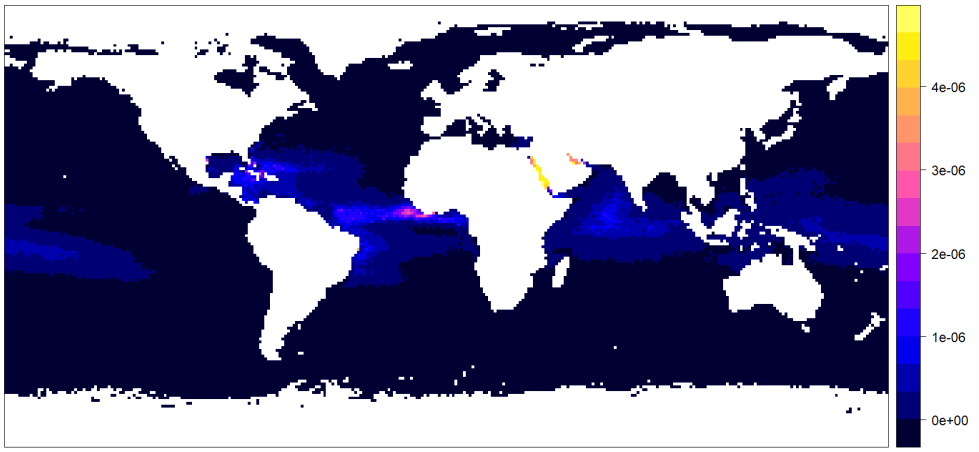

Figure 7 Estimated probability origin of the tooth sample T26 (attributed to the species *P. pristis* and with documental geographic origin: "Madras, India") calculated from the values of the oxygen isotopes. Yellow colours showed the highest probability and dark blue the lowest.

Among the 24 individuals without documental geographic origin (Table 1) assigned to isoscapes with high probability, one *P. zijsron* individual (T35) was assigned to high-latitude or Mediterranean isoscapes, deserving special attention (Figure 8); two *P. zijsron* individuals (F20 and T32) were precisely assigned to the Persian Gulf (Figure 9); three *P. pristis* (F11, F12 and T37) and one *P. zijsron* (C73) to the Red Sea and the Persian Gulf (Figure 10); two *P. zijsron* (C70 and C76) to the Red Sea and Central-Western Atlantic Ocean (Figure 11).

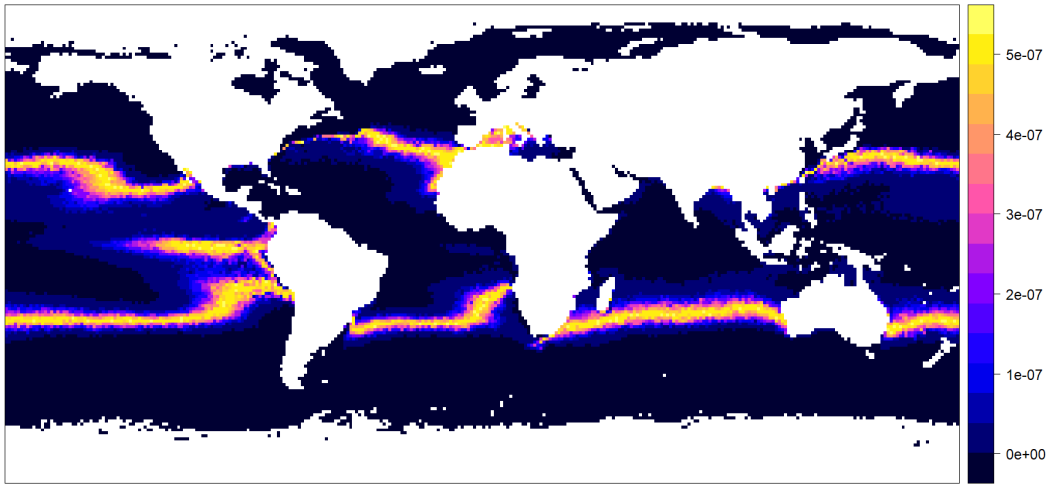

Figure 8 Estimated probability origin of the tooth sample T35 (attributed to the species *P. zijsron* and without documental geographic origin) calculated from the values of the oxygen isotopes. Yellow colours showed the highest probability and dark blue the lowest.

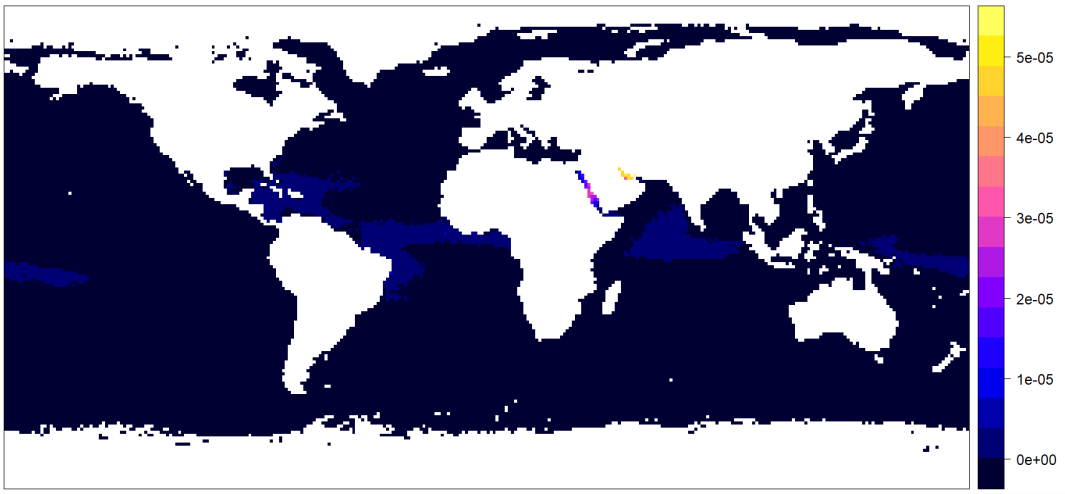

Figure 9 Estimated probability origin of the tooth sample F20 (attributed to the species *P. zijsron* and without documental geographic origin) calculated from the values of the oxygen isotopes. Yellow colours showed the highest probability and dark blue the lowest.

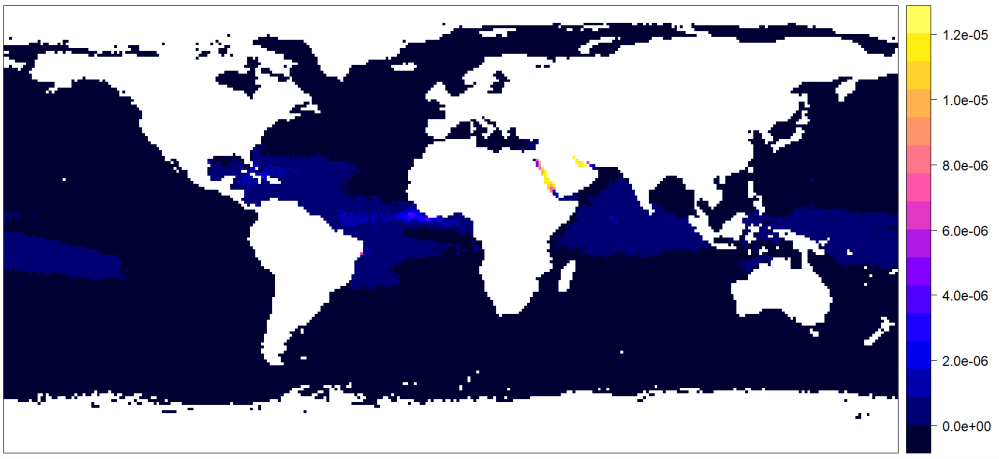

Figure 10 Estimated probability origin of the tooth sample F11 (attributed to the species *P. pristis* and without documental geographic origin) calculated from the values of the oxygen isotopes. Yellow colours showed the highest probability and dark blue the lowest.

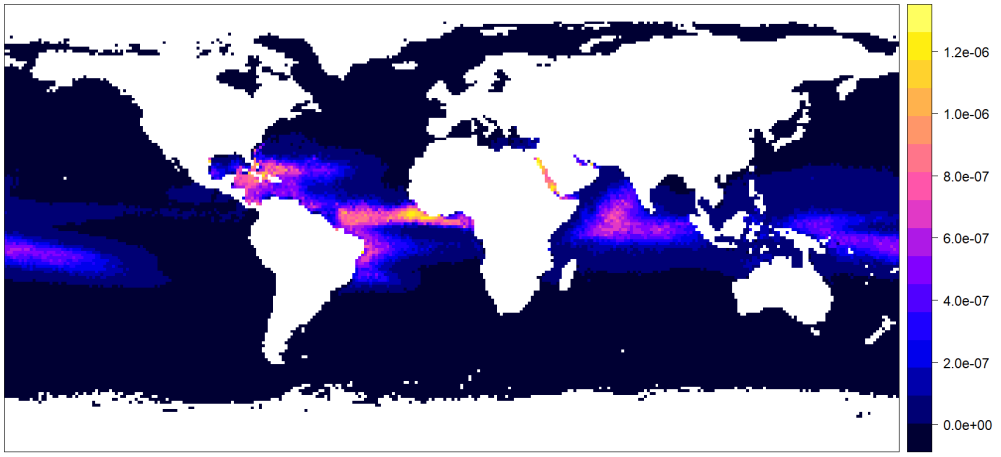

Figure 11 Estimated probability origin of the tooth sample C70 (attributed to the species *P. zijsron* and without documental geographic origin) calculated from the values of the oxygen isotopes. Yellow colours showed the highest probability and dark blue the lowest.

The remaining 15 individuals appeared of uncertain origin because they were assigned contemporaneously to the Indian, Pacific, Atlantic, and Mediterranean isoscapes: however, the Pacific and Atlantic origin can be excluded for eleven *P. zijsron* individuals (F3, F6, F18, T28, T29, T30, T31, T36, Pi63, C69 and C75) since the species was not distributed in these areas. The two individuals belonging to *A. cuspidata* (F9 and F10) were most likely of Indo-Pacific origin (Figure 12) while this area of origin can be excluded for the two individuals of *P. pectinata* (F15 and T34) since the species was not distributed in these areas.

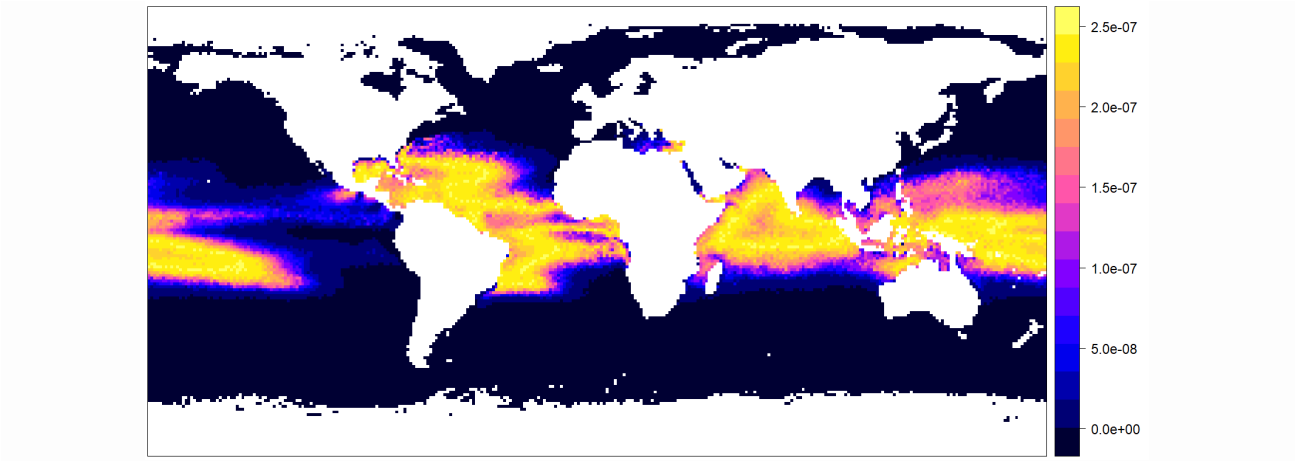

Figure 12 Estimated probability origin of the tooth sample F9 (attributed to the species *A. cuspidata* and without documental geographic origin) calculated from the values of the oxygen isotopes. Yellow colours showed the highest probability and dark blue the lowest.

Overall, among 32 individuals assigned with high probability by isotopic analyses (Table 2), 12 were assigned to a given isoscape/area (considering the Red Sea and the Persian Gulf of the same isoscape/area). The other 20 individual rostra were assigned with uncertainty (two-four isoscapes/areas) and geographical origin can be traced by considering the species distribution. Only three individuals out of eight showed a consistency between documental and isotopic origin, while two individuals showed a documental origin coherent with at least one of the multiple origins obtained with isotope analysis.

Table 2. Summary of isotopic tooth analysis of the 35 individual rostra of historical sawfish

Isoscape/area	Number of individuals with high-probability isotopic origin	Number of individuals with concordant high-probability isotopic origin and documental origin
Mediterranean	**1** (T35)	
Red Sea, Red Sea/Persian Gulf	**11** (C72, C73 C74, F11, F12, F14, F20, P59, T26, T32, T37)	**2** (F14, C72)
Red Sea/CW Atlantic	**3** (Picci84, C70, C76)	**1** (Picci84)
Indian Ocean, Pacific Ocean, Atlantic Ocean, and the Mediterranean Sea	**17** (C69, C71, C75, F2, F3, F6, F9, F10, F15, Pi63, F18, T28, T29, T30, T31, T34, T36)	**2** (F2, C71)

Three individuals (F16, F17 and F19) without documental origin, showed low probabilities to be assigned.

3.4 Discussion

The alarming global reduction in sawfish populations led the scientific community to address huge and widespread research efforts to better determine the status of the species in poorly studied regions and to promote biological studies to provide the data needed to establish management and restoration plans (Dulvy et al., 2014, 2016) for the recovery of the remaining populations in the world.

Within this framework, the present chapter effectively contributes to increase the knowledge of historical ecology of sawfishes, to retrieve with statistical confidence the geographical origin of individuals (given their ascertained species by an integrated morphometric and molecular taxonomic analysis of rostra available in museum and private collections achieved in the Chapter 2) and to test the historical presence of this tropical/subtropical elasmobranchs in the temperate Mediterranean Sea. This multidisciplinary approach combined the species taxonomy of historical rostra together with the analysis of stable isotopes of carbon and oxygen mineralized into calcium carbonate matrix of rostral teeth (Combes, Cazalbou, & Rey, 2016) and the estimate of probability of the sample's likelihood to be assigned to the most probable origin (Torniainen et al., 2017; Bird et al., 2018). The new technique permitted the identification of the high-probability habitat preferences of these benthopelagic elasmobranchs in about 50% of the analysed specimens. Fifteen out of 32 individual rostra have been assigned to a given geographical area of origin with high probability, according to the matching between the estimated $\delta^{18}O$ of each tooth and the values of the corresponding $\delta^{18}O$ isoscapes. Among them, 12 individuals were precisely assigned to the Mediterranean (one individual belonging to *P. zijsron* with unknown documental origin whose rostrum is musealized at the Natural history museum of Trieste) and to the Red Sea and Persian Gulf (11 individuals of *P. zijsron* and *P. pristis*; see Table S1, Table 1 and Table 2). The geographical assignment of other 20 individuals was not univocally achieved by isotope analysis since two to four isoscapes were matched by the individual $\delta^{18}O$ composition. Thus, the integration with the species distribution data was used to assign most of them. Most of the geographical assignments were consistent with the species distribution (Faria et al., 2013; Dulvy et al., 2014). Indeed, *P. pristis* could inhabit many areas such as Eastern Atlantic, Western Atlantic, Eastern Pacific, and Indo-West Pacific (Faria et al., 2013); *P. pectinata* could be found in West and East Atlantic subtropical waters and the other two species have an Indo West-Pacific distribution especially occur from the Red Sea to Australian coast (Faria et al., 2013). Several individuals of *P. zijsron* were assigned to the Persian Gulf or the Red Sea with high probabilities,

while others seemed to come from the African coasts of the Indian Ocean and from the North Australian coasts. A remarkable result was observed for the specimen T35, in which our estimated temperatures corresponded to too high latitude or within the Mediterranean Sea. The presence of the species has not been documented in the Mediterranean Sea so far (Faria et al., 2013), but according to the isotope composition, the sample had a high probability of being native of the Adriatic, Tyrrhenian, or west Mediterranean waters. In addition, all the five individuals of *P. pristis* appeared to occur in the Persian Gulf or in the Red Sea with high probabilities. The two specimens of *P. pectinata* had a high probability to come from the Central Atlantic coasts and the Mediterranean Sea, excluding the Indo-Pacific areas. Finally, the two *A. cuspidata* most likely were of Indo-Pacific origin. It is also relevant to be noticed that in five out of eight cases, the isotope composition is consistent with the documental geographic origin of rostra proving the adequateness of this technique for studies in historical biogeography and ecology of marine cartilaginous fish.

The isotope composition analysis was previously used to discriminate from different habitats using shark teeth (Carlisle et al., 2012), vertebral centra (Christiansen et al., 2014), or fish otoliths (Sisma-Ventura et al., 2019; Willmes et al., 2019). Recently, oxygen isotope was used to reconstruct paleoenvironmental conditions (Kocsis, Vennemann, & Fontignie, 2007; Sisma-Ventura et al., 2019; Willmes et al., 2019; Séon et al., 2020) or to calculate the temperature of the seas to identify the origin of the samples (Bigg & Rohling, 2000; Vennemann et al., 2001; Séon et al., 2020). Instead, carbon isotope could evaluate trophic food web (Rubenstein & Hobson, 2004; Logan & Lutcavage, 2010; Reum, Williams, & Harvey, 2017), and could track migration pattern of different marine species (Trueman, Mackenzie, & Palmer, 2012). Ancient sawfish species were tested for the oxygen and carbon composition of fossil vertebras (Amiot et al., 2010). Studies on different marine species, such as sharks or teleosts, took into account the oxygen and carbon istope composition in teeth structures (Vennemann et al., 2001; Guy et al., 2018) or in other tissues (Borrell et al., 2011; Trueman, Mackenzie, & Palmer, 2012) to evaluate the migratory patterns.

The low variation of values in the oxygen and carbon isotope composition of our samples led to assume that they dwelled in areas with a similar isotope composition. Moreover, our samples belong to the same range of sharks and fishes from the central Atlantic and Indian Ocean. Globally, the estimation of the temperature was a good proxy to the overall distribution. However, it was not reliable enough to express the real provenance of samples since, considering the average value of $\delta^{18}O_W$VSMOW, it was not possible to punctually refer to a specific coastal area. A technical controversial issue deserve our attention in doing these comparisons: in literature, samples were often treated with NaClO or H_2O_2 to remove the organic matrix that usually occurs in teeth (Ventresca

Miller et al., 2018). Our samples were not treated with any reagent because analytical errors and higher isotope values may increase with treatment, caused by the atmospheric CO_2 that may be incorporated during the treatment (Crowley & Wheatley, 2014). According to Crowley & Wheatley (2014), samples treated with H_2O_2 are comparable with the not-treated ones (Guy et al., 2018; Löffler et al., 2019; Killam et al., 2020; Séon et al., 2020; Chung et al., 2021). Although the NaClO treatment gives different values from not-treated ones, we used for comparison only data from non-recent studies (Amiot et al., 2010, Vennemann et al., 2001) that used different instruments, allowing the comparison between processed and unprocessed data.

Further analyses are required in these perspectives to assess the real provenance of all the samples. Indeed, using the organic carbonate compound of tooth apatite it was possible to establish the provenance of sharks (Bird et al., 2018), thus the same workflow should be expected to be effective for sawfishes. Moreover, the results from the Oxygen contained in the inorganic matrix and the Carbon from the organic one could be combined to strengthen the estimation of geographical origin of each sample (Torniainen et al., 2017). Despite their ecological importance, sawfish species are particularly vulnerable due to their sensitive life histories. Therefore, the identification of sawfish real distribution is mandatory to assess correct and appropriate conservation measures preventing their decline.

3.5 References

Abrantes, K. G., & Barnett, A. (2011). Intrapopulation variations in diet and habitat use in a marine apex predator, the broadnose sevengill shark Notorynchus cepedianus. *Mar. Ecol. Prog. Ser.* **442**, 133–148.

Amiot, R., Wang, X., Lécuyer, C., Buffetaut, E., Boudad, L., Cavin, L., Ding, Z., Fluteau, F., Kellner, A. W. A., Tong, H., & Zhang, F. (2010). Oxygen and carbon isotope compositions of middle Cretaceous vertebrates from North Africa and Brazil: Ecological and environmental significance. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **297**, 439–451.

Bigg, G. R., & Rohling, E. J. (2000). An oxygen isotope data set for marine waters. *J. Geophys. Res. Oceans* **105**, 8527–8535.

Bird, C. S., Veríssimo, A., Magozzi, S., Abrantes, K. G., Aguilar, A., Al-Reasi, H., Barnett, A., Bethea, D. M., Biais, G., Borrell, A., Bouchoucha, M., Boyle, M., Brooks, E. J., Brunnschweiler, J., Bustamante, P., Carlisle, A., Catarino, D., Caut, S., Cherel, Y., … & Trueman, C. N. (2018). A global perspective on the trophic geography of sharks. *Nat. Ecol. Evol.* **2**, 299–305.

Boecklen, W. J., Yarnes, C. T., Cook, B. A., & James, A. C. (2011). On the Use of Stable Isotopes in Trophic Ecology. *Annu. Rev. Ecol. Evol. Syst.* **42**, 411–440.

Borrell, A., Aguilar, A., Gazo, M., Kumarran, R. P., & Cardona, L. (2011). Stable isotope profiles in whale shark (*Rhincodon typus*) suggest segregation and dissimilarities in the diet depending on sex and size. *Environ. Biol. Fishes* **92**, 559–567.

Carlisle, A. B., Kim, S. L., Semmens, B. X., Madigan, D. J., Jorgensen, S. J., Perle, C. R., Anderson, S. D., Chapple, T. K., Kanive, P. E., & Block, B. A. (2012). Using stable isotope analysis to understand the migration and trophic ecology of northeastern Pacific white sharks (*Carcharodon carcharias*). *PLoS ONE* **7**.

Christiansen, H. M., Hussey, N. E., Wintner, S. P., Cliff, G., Dudley, S. F. J., & Fisk, A. T. (2014). Effect of sample preparation techniques for carbon and nitrogen stable isotope analysis of

hydroxyapatite structures in the form of elasmobranch vertebral centra. *Rapid Commun. Mass Spectrom.* **28**, 448–456.

Chung, M.-T., Chen, C.-Y., Shiao, J.-C., Shirai, K., & Wang, C.-H. (2021). Metabolic proxy for cephalopods: Stable carbon isotope values recorded in different biogenic carbonates. *Methods Ecol. Evol.* **12**, 1648–1657.

Combes, C., Cazalbou, S., & Rey, C. (2016). Apatite biominerals. *Minerals* **6**, 1–25.

Crowley, B. E., & Wheatley, P. V. (2014). To bleach or not to bleach? Comparing treatment methods for isolating biogenic carbonate. *Chem. Geol.* **381**, 234–242.

Dulvy, N. K., Davidson, L. N. K., Kyne, P. M., Simpfendorfer, C. A., Harrison, L. R., Carlson, J. K., & Fordham, S. V. (2016). Ghosts of the coast: Global extinction risk and conservation of sawfishes. *Aquat. Conserv. Mar. Freshw. Ecosyst.* **26**, 134–153.

Dulvy, N. K., Fowler, S. L., Musick, J. A., Cavanagh, R. D., Kyne, P. M., Harrison, L. R., Carlson, J. K., Davidson, L. N., Fordham, S. V, Francis, M. P., Pollock, C. M., Simpfendorfer, C. A., Burgess, G. H., Carpenter, K. E., Compagno, L. J., Ebert, D. A., Gibson, C., Heupel, M. R., Livingstone, S. R., Sanciangco, J. C., Stevens, J. D., Valenti, S., & White, W. T. (2014). Extinction risk and conservation of the world's sharks and rays. *eLife* **3**, 1–34.

Faria, V. V., McDavitt, M. T., Charvet, P., Wiley, T. R., Simpfendorfer, C. A., & Naylor, G. J. P. (2013). Species delineation and global population structure of Critically Endangered sawfishes (Pristidae). *Zool. J. Linn. Soc.* **167**, 136–164.

Ferretti, F., Morey Verd, G., Seret, B., Sulić Šprem, J., & Micheli, F. (2016). Falling through the cracks: the fading history of a large iconic predator. *Fish Fish.* **17**, 875–889.

Field I.C., Meekan M.G. & Bradshaw C.J.A. (2009). Development of non-lethal methods for determining age and habitat use of sawfishes from northern Australia. Final Report.

Grossman, E. L., & Ku, T.-L. (1986). Oxygen and carbon isotope fractionation in biogenic aragonite: Temperature effects. *Chem. Geol. Isot. Geosci. Sect.* **59**, 59–74.

Guy, S.V., Thomas, T., Irit, Z., Andreas, P., Dorit, S., Omri, L., Ayelet, G., & Guy, B.-O. (2018). Tooth oxygen isotopes reveal Late Bronze Age origin of Mediterranean fish aquaculture and trade. *Sci. Rep.* **8**, 14086.

Hegg, J. C., Graves, B., & Fisher, C. M. (2021). Sawfish, read in tooth and saw: Rostral teeth as endogenous chemical records of movement and life-history in a critically endangered species. *Aquat. Conserv. Mar. Freshw. Ecosyst.* **31**(9), 2334–2347.

Hijmans, R. J., Etten, J. van, Sumner, M., Cheng, J., Baston, D., Bevan, A., Bivand, R., Busetto, L., Canty, M., Fasoli, B., Forrest, D., Ghosh, A., Golicher, D., Gray, J., Greenberg, J. A., Hiemstra, P., Hingee, K., Ilich, A., Geosciences, I. for M. A., Karney, C., Mattiuzzi, M., Mosher, S., Naimi, B., Nowosad, J., Pebesma, E., Lamigueiro, O. P., Racine, E. B., Rowlingson, B., Shortridge, A., Venables, B., & Wueest, R. (2021). raster: Geographic Data Analysis and Modeling.

Hobson, K. A. (1999). Tracing origins and migration of wildlife using stable isotopes: a review. *Oecologia* **120**, 314–326.

Hollensead, L. D., Dean Grubbs, R., Carlson, J. K., & Bethea, D. M. (2018). Assessing residency time and habitat use of juvenile smalltooth sawfish using acoustic monitoring in a nursery habitat. *Endanger. Species Res.* **37**, 119–131.

Hollensead, L. D., Grubbs, R. D., Carlson, J. K., & Bethea, D. M. (2016). Analysis of fine-scale daily movement patterns of juvenile *Pristis pectinata* within a nursery habitat. *Aquat. Conserv. Mar. Freshw. Ecosyst.* **26**, 492–505.

Hussey, N. E., MacNeil, M. A., McMeans, B. C., Olin, J. A., Dudley, S. F. J., Cliff, G., Wintner, S. P., Fennessy, S. T., & Fisk, A. T. (2014). Rescaling the trophic structure of marine food webs. *Ecol. Lett.* **17**, 239–250.

Killam, D., Thomas, R., Al-Najjar, T., & Clapham, M. (2020). Interspecific and Intrashell Stable Isotope Variation Among the Red Sea Giant Clams. *Geochem. Geophys. Geosystems* **21**, e2019GC008669.

Kocsis, L., Vennemann, T. W., & Fontignie, D. (2007). Migration of sharks into freshwater systems during the Miocene and implications for Alpine paleoelevation. *Geology* **35**, 451–454.

Layman, C. A., Araujo, M. S., Boucek, R., Hammerschlag-Peyer, C. M., Harrison, E., Jud, Z. R., Matich, P., Rosenblatt, A. E., Vaudo, J. J., Yeager, L. A., Post, D. M., & Bearhop, S. (2012). Applying stable isotopes to examine food-web structure: an overview of analytical tools. *Biol. Rev.* **87**, 545–562.

Lécuyer, C., Amiot, R., Touzeau, A., & Trotter, J. (2013). Calibration of the phosphate δ18O thermometer with carbonate-water oxygen isotope fractionation equations. *Chem. Geol.* **347**, 217–226.

Löffler, N., Fiebig, J., Mulch, A., Tütken, T., Schmidt, B. C., Bajnai, D., Conrad, A. C., Wacker, U., & Böttcher, M. E. (2019). Refining the temperature dependence of the oxygen and clumped isotopic compositions of structurally bound carbonate in apatite. *Geochim. Cosmochim. Acta* **253**, 19–38.

Logan, J. M., & Lutcavage, M. E. (2010). Stable isotope dynamics in elasmobranch fishes. *Hydrobiologia* **644**, 231–244.

Ma, C., Vander Zanden, H. B., Wunder, M. B., & Bowen, G. J. (2020). assignR: An r package for isotope-based geographic assignment. *Methods Ecol. Evol.* **11**, 996–1001.

MacNeil, M. A., Skomal, G. B., & Fisk, A. T. (2005). Stable isotopes from multiple tissues reveal diet switching in sharks. *Mar. Ecol. Prog. Ser.* **302**, 199–206.

Matich, P. M., Heithaus, M. R. H. R., & Layman, C. A. L. A. (2010). Size-based variation in intertissue comparisons of stable carbon and nitrogen isotopic signatures of bull sharks (*Carcharhinus leucas*) and tiger sharks (*Galeocerdo cuvier*). *Can. J. Fish. Aquat. Sci.* **67**, 877–885.

Pearson, R. M., van de Merwe, J. P., & Connolly, R. M. (2020). Global oxygen isoscapes for barnacle shells: Application for tracing movement in oceans. *Sci. Total Environ.* **705**, 135782.

Peterson, B. J., & Fry, B. (1987). Stable Isotopes in Ecosystem Studies. *Annu. Rev. Ecol. Syst.* **18**, 293–320.

Poulakis, G. R., & Seitz, J. C. (2004). Recent occurrence of the smalltooth sawfish, Pristis pectinata (Elasmobranchiomorphi: Pristidae), in Florida Bay and the Florida Keys, with comments on sawfish ecology. *Fla. Sci.* **67**, 27–35.

RStudio Team (2021). RStudio: Integrated Development Environment for R. RStudio, PBC, Boston. http://www.rstudio.com

Ramos, R., & González-Solís, J. (2012). Trace me if you can: the use of intrinsic biogeochemical markers in marine top predators. *Front. Ecol. Environ.* **10**, 258–266.

Reum, J. C. P., Williams, G. D., & Harvey, C. J. (2017). Chapter Five - Stable Isotope Applications for Understanding Shark Ecology in the Northeast Pacific Ocean. In S. E. Larson & D. Lowry (Eds.), *Adv. Mar. Biol.* Vol. 77, pp. 149–178. Elsevier Ltd.

Rubenstein, D. R., & Hobson, K. A. (2004). From birds to butterflies: Animal movement patterns and stable isotopes. *Trends Ecol. Evol.* **19**, 256–263.

Schmidt, G. A. (1999). Forward modeling of carbonate proxy data from planktonic foraminifera using oxygen isotope tracers in a global ocean model. *Paleoceanography* **14**, 482–497.

Séon, N., Amiot, R., Martin, J. E., Young, M. T., Middleton, H., Fourel, F., Picot, L., Valentin, X., & Lécuyer, C. (2020). Thermophysiologies of Jurassic marine crocodylomorphs inferred from the oxygen isotope composition of their tooth apatite. *Philos. Trans. R. Soc. B Biol. Sci.* **375**, 20190139.

Sisma-Ventura, G., Tütken, T., Peters, S. T. M., Bialik, O. M., Zohar, I., & Pack, A. (2019). Past aquatic environments in the Levant inferred from stable isotope compositions of carbonate and phosphate in fish teeth. *PLOS ONE* **14**, e0220390.

Torniainen, J., Lensu, A., Vuorinen, P. J., Sonninen, E., Keinänen, M., Jones, R. I., Patterson, W. P., & Kiljunen, M. (2017). Oxygen and carbon isoscapes for the Baltic Sea: Testing their applicability in fish migration studies. *Ecol. Evol.* **7**, 2255–2267.

Trueman, C. N., Mackenzie, K. M., & Palmer, M. R. (2012). Identifying migrations in marine fishes through stable-isotope analysis. *J. Fish Biol.* **81**, 826–847.

Vennemann, T. W., Hegner, E., Cliff, G., & Benz, G. W. (2001). Isotopic composition of recent shark teeth as a proxy for environmental conditions. *Geochim. Cosmochim. Acta* **65**, 1583–1599.

Ventresca Miller, A., Fernandes, R., Janzen, A., Nayak, A., Swift, J., Zech, J., Boivin, N., & Roberts, P. (2018). Sampling and pretreatment of tooth enamel carbonate for stable carbon and oxygen isotope analysis. *J. Vis. Exp.* **138**, e58002.

Willmes, M., Lewis, L. S., Davis, B. E., Loiselle, L., James, H. F., Denny, C., Baxter, R., Conrad, J. L., Fangue, N. A., Hung, T.-C., Armstrong, R. A., Williams, I. S., Holden, P., & Hobbs, J. A. (2019). Calibrating temperature reconstructions from fish otolith oxygen isotope analysis for

California's critically endangered Delta Smelt. *Rapid Commun. Mass Spectrom.* **33**, 1207–1220.

Wueringer, B. E., Squire, L., & Collin, S. P. (2009). The biology of extinct and extant sawfish (Batoidea: Sclerorhynchidae and Pristidae). *Rev. Fish Biol. Fish.* **19**, 445–464.

Yan, H. F., Kyne, P. M., Jabado, R. W., Leeney, R. H., Davidson, L. N. K., Derrick, D. H., Finucci, B., Freckleton, R. P., Fordham, S. V., & Dulvy, N. K. (2021). Overfishing and habitat loss drive range contraction of iconic marine fishes to near extinction. *Sci. Adv.* **7**, eabb6026.

3.6 Supplementary material

Table S1. Reference dataset for Carbon and Oxygen composition of carbonate and phosphate, considering subdivision in macro-groups.

Geographical area	Taxa	$\delta^{13}C$ VPDB	$\delta^{18}O_C$ VPDB	$\delta^{18}O_C$ VSMOW	$\delta^{18}O_P$ VSMOW	Reference
Artic Ocean	*Somniosus microcephalus*	-9	4.11	35.1	25.6	Vennemann et al. (2001)
	Somniosus microcephalus	-8.6	2.85	33.8	24.7	
	Somniosus pacificus	-10.8	1.01	31.9	24.9	
Indian Ocean	*Carcharias taurus*	-5.5	0.52	31.4	22.6	
	Carcharias taurus	-6.4	0.04	30.9	22.7	
	Carcharias taurus	-6.3	-0.54	30.3	22.8	
	Carcharias taurus	-2.7	2.37	33.3	22.7	
	Carcharias taurus	-4.3	5.86	36.9	22.3	
	Carcharias taurus	-2.9	2.08	33	22.5	
	Carcharias taurus	-5.6	1.11	32	22.2	
	Carcharias taurus	1.6	0.81	31.7	22.4	
	Carcharias taurus	-5.1	1.3	32.2	22.1	
	Carcharias taurus	2.8	1.88	32.8	22.9	
	Carcharias taurus	-4.5	2.56	33.5	22.5	
	Carcharias taurus	1.6	1.69	32.6	22.6	
	Carcharias taurus	-5.1	0.81	31.7	22.8	
	Carcharias taurus	2.1	0.52	31.4	22.6	
	Carcharias taurus	-4.9	1.3	32.2	22.6	
	Carcharodon carcharias	-3	-0.06	30.8	22.4	
	Carcharodon carcharias	-5.6	1.3	32.2	23.1	
	Carcharodon carcharias	-3.2	-0.25	30.6	22.8	
	Carcharodon carcharias	-0.9	0.72	31.6	22.6	
	Carcharodon carcharias	-3.3	-0.45	30.4	22.3	
	Carcharodon carcharias	4.8	3.14	34.1	25	
	Carcharodon carcharias	-4.1	1.01	31.9	22.8	
	Carcharodon carcharias	4	2.85	33.8	24.7	
	Carcharodon carcharias	-2.9	0.43	31.3	22.2	
	Carcharodon carcharias	-4.6	0.14	31	21.9	
	Carcharodon carcharias	1.8	1.4	32.3	22.7	
	Carcharodon carcharias	-3.5	-0.06	30.8	22.3	
	Carcharodon carcharias	2.3	1.01	31.9	22.8	
	Carcharodon carcharias	-4.3	0.14	31	21.9	
	Carcharodon carcharias	2.3	1.79	32.7	23.6	
	Carcharodon carcharias	-3.8	0.33	31.2	22.1	
	Carcharodon carcharias	-3.1	0.23	31.1	23	
	Isurus oxyrhincus	-2.1	-1.03	29.8	23.2	
	Isurus oxyrhincus	-1.8	0.23	31.1	22.7	
	Carcharhinus limbatu	-1.7	-1.03	29.8	22.5	
	Carcharhinus limbatu	-2.1	0.23	31.1	22.8	

	Carcharhinus obscurus	-3.4	0.04	30.9	21.5	
	Galeocerdo cuvier	-4.1	-0.45	30.4	21.4	
	Galeocerdo cuvier	-3.4	1.69	32.6	22.7	
	Galeocerdo cuvier	-3.6	0.81	31.7	22.7	
	Galeocerdo cuvier	-2.1	-0.06	30.8	21.5	
	Galeocerdo cuvier	-3.2	-2.19	28.6	22.1	
	Galeocerdo cuvier	-2.2	-0.74	30.1	21.7	
	Galeocerdo cuvier	-3.9	-3.16	27.6	18.5	
	Sphyrna mokarran	-0.6	0.52	31.4	22	
	Sphyrna mokarran	-2.3	0.23	31.1	21.5	
Atlantic SW	Ceratodontiformes indet.	-8	-3.94	26.8	20.2	Amiot et al. (2010)
	Ceratodontiformes indet.	-6.9	-4.91	25.8	19.5	
Atlantic C	cf. *Neoceratodus africanus*	-10.3	-5.3	25.4	19.1	
	cf. *Neoceratodus africanus*	-10.3	-4.62	26.1	19.9	
	cf. *Neoceratodus africanus*	-11.1	-5.78	24.9	20.2	
Atlantic C	*Onchopristis numidus*	-8.2	-7.24	23.4	19.6	
	Onchopristis numidus	-6.2	-7.63	23	18.8	
	Onchopristis numidus	-8.7	-5.88	24.8	18.8	
	Onchopristis numidus	-6.8	-7.53	23.1	19.6	
Atlantic C	Amiiformes indet.	-2.62	-5.17	25.53	20.3	Séon et al. (2020)
	Amiiformes indet.	-1.4	-6.16	24.51	20.7	
	Gyrodus cuvieiri	3.2	-4.23	26.5	19.9	
	Gyrodus cuvieiri	1.4	-4.52	26.2	20.5	
	Gyrodus cuvieiri	0.5	-6.07	24.6	19.6	
	Hypsocormus sp.	-1.2	-5.3	25.4	21.8	
	Hypsocormus sp.	-1.6	-5.49	25.2	20.7	
	Lepidotes sp.	-1.4	-4.71	26	20.7	
	Lepidotes sp.	-1	-4.52	26.2	20.3	
	Lepidotes sp.	-0.6	-5.1	25.6	20.2	
	Hypsocormus sp.	-1.6	-5.78	24.9	19.7	
	Hypsocormus sp.	-4.4	-2.97	27.8	20.5	
	Hypsocormus sp.	-1.9	-4.71	26	20.2	
	Hypsocormus sp.	-2.4	-5.3	25.4	20.5	
Atlantic C	*Asteracanthus* sp.	-1.2	-5.78	24.9	20	
	Asteracanthus sp.	-1.9	-5.68	25	19.8	
	Asteracanthus sp.	7.7	-5.39	25.3	18.6	
	Hybodus obtusus	-4.1	-5.3	25.4	20.4	
	Hybodus obtusus	-4	-3.84	26.9	21.3	
	Hybodus obtusus	-5.2	-5.88	24.8	20.1	
	Hybodus obtusus	-3.8	-5.2	25.5	21	
	Hybodus obtusus	-4.9	-4.33	26.4	21	
	Ischyodus sp.	-1.4	-5.59	25.1	20.3	
	Ischyodus sp.	-0.3	-5.88	24.8	20.5	
	Ischyodus sp.	-0.4	-4.33	26.4	20.9	

Mediterranean	*Sparus aurata*	-1.8	1.59	32.5	22.5	Guy et al. (2018)
	Sparus aurata	-2.1	0.72	31.6	22.6	
	Sparus aurata	-3	0.23	31.1	22.6	
	Sparus aurata	-0.1	1.49	32.4	23.3	
	Sparus aurata	0.6	2.56	33.5	23.2	
	Sparus aurata	-0.5	3.24	34.2	23.2	
	Sparus aurata	0.6	2.56	33.5	23.2	
	Sparus aurata	0.1	1.4	32.3	23.4	
	Sparus aurata	0.1	2.37	33.3	23.4	
	Sparus aurata	-0.7	1.49	32.4	23.3	
	Sparus aurata	0.5	2.27	33.2	23.2	
	Sparus aurata	0.3	1.2	32.1	23.2	
	Sparus aurata	0.3	1.49	32.4	23.3	
	Sparus aurata	0.6	2.56	33.5	23.3	
	Sparus aurata	0	2.46	33.4	23.4	
	Sparus aurata	-6.2	-0.35	30.5	21	
	Sparus aurata	-6.7	-5.78	24.9	21.1	
	Sparus aurata	-6.2	-0.35	30.5	21.7	
	Sparus aurata	-5.2	-0.06	30.8	21.7	
	Sparus aurata	3.8	3.43	34.4	23.4	
Indian Ocean	*Carcharodon megalodon*	-3.97	1.25	32.16	23.06	Löffler et al. (2019)
Atlantic_N	*Carcharodon megalodon*	1.38	2.98	33.95	24.85	
	Carcharodon megalodon	-3.03	0.91	31.8	22.7	
Red Sea	Cephalopoda	0.5	1.2	32.1	23	Chung et al. (2021)
	Cephalopoda	0.4	-0.01	30.85	21.75	
	Cephalopoda	0.5	0	30.86	21.76	
	Cephalopoda	0.7	0.19	31.06	21.96	
	Cephalopoda	0.8	0.04	30.9	21.8	
	Cephalopoda	0.23	1.3	32.2	23.1	
	Cephalopoda	2.12	0.95	31.84	22.74	
	Cephalopoda	1.5	0.77	31.65	22.55	
	Cephalopoda	2.22	0.99	31.88	22.78	
	Cephalopoda	1.54	0.89	31.78	22.68	
	Cephalopoda	1.71	1.28	32.18	23.08	
	Cephalopoda	1.47	1.33	32.23	23.13	
	Cephalopoda	1.63	1.47	32.38	23.28	
	Cephalopoda	1.36	1.77	32.68	23.58	
	Cephalopoda	-3	-0.81	30.03	20.93	
	Cephalopoda	-2.85	-0.48	30.37	21.27	
	Cephalopoda	-2.23	0.1	30.96	21.86	
	Cephalopoda	-7.15	0.76	31.64	22.54	
	Cephalopoda	-6.79	0.54	31.42	22.32	
	Cephalopoda	-6.37	0.4	31.27	22.17	
	Cephalopoda	-4.4	-0.6	30.24	21.14	
	Cephalopoda	-4.8	-0.4	30.45	21.35	

	Cephalopoda	-5.4	-0.9	29.93	20.83	
	Cephalopoda	-5.6	-0.8	30.04	20.94	
	Cephalopoda	-1.2	2.1	33.02	23.92	
	Cephalopoda	-1.5	1.7	32.61	23.51	
	Cephalopoda	-1.1	2.2	33.13	24.03	
	Cephalopoda	-1	1.6	32.51	23.41	
	Cephalopoda	-0.5	1.8	32.72	23.62	
	Cephalopoda	-1.4	1.3	32.2	23.1	
	Cephalopoda	-0.5	3	33.95	24.85	
	Cephalopoda	-0.2	3	33.95	24.85	
	Cephalopoda	-1.6	1.6	32.51	23.41	
	Cephalopoda	-1.815	0.5	31.38	22.28	
	Cephalopoda	-1.455	0.39	31.26	22.16	
	Cephalopoda	-1.685	0.57	31.44	22.34	
	Cephalopoda	-1.895	1.36	32.26	23.16	
	Cephalopoda	-2.645	1.51	32.41	23.31	
	Cephalopoda	-1.485	0.84	31.72	22.62	
	Cephalopoda	-2.225	0.99	31.88	22.78	
	Cephalopoda	-2.55	0.41	31.28	22.18	
	Cephalopoda	-2.895	0.54	31.41	22.31	
	Cephalopoda	-3.38	1.28	32.18	23.08	
	Cephalopoda	-4.3	0.09	30.95	21.85	
	Cephalopoda	-2.65	0.85	31.74	22.64	
Red Sea	Bivalvia	2.37	0.58	31.45	22.35	Killam et al. (2020)
	Bivalvia	1.95	0.48	31.36	22.26	
	Bivalvia	1.96	0.58	31.46	22.36	
	Bivalvia	1.96	0.56	31.44	22.34	
	Bivalvia	2.07	0.85	31.74	22.64	
Mediterranean	Bivalvia	1.62	1.23	32.13	23.03	
	Bivalvia	1.6	0.97	31.86	22.76	
	Bivalvia	1.47	1.1	31.99	22.89	
	Bivalvia	1.42	1.36	32.26	23.16	
	Bivalvia	1.44	1.48	32.39	23.29	
	Bivalvia	1.56	1.42	32.32	23.22	
	Bivalvia	1.6	1.23	32.13	23.03	
	Bivalvia	1.36	0.91	31.8	22.7	
	Bivalvia	1.34	1.16	32.05	22.95	
	Bivalvia	3.13	1.69	32.6	23.5	
	Bivalvia	3.04	1.39	32.3	23.2	
	Bivalvia	2.88	2.23	33.16	24.06	
	Bivalvia	2.96	2.09	33.01	23.91	
Red Sea	Bivalvia	1.77	0.62	31.5	22.4	
	Bivalvia	1.79	0.89	31.78	22.68	
	Bivalvia	1.75	0.79	31.67	22.57	
	Bivalvia	1.8	0.88	31.77	22.67	
	Bivalvia	1.76	1.09	31.98	22.88	

Table S2. Estimated temperature using oxygen isotopes from carbonate and phosphate for different seas, using the coordinate of Indian Ocean (IO), Red Sea (RS), Mediterranean Sea (med) and Central Atlantic Ocean (CAO), and values of $\delta^{18}O_W$VMSOW calculated using the lowest, mean, and highest possible temperature for sawfish.

Samples	T°C IO ($\delta^{18}O_P$)	T°C IO ($\delta^{18}O_C$)	T°C RS ($\delta^{18}O_P$)	T°C RS ($\delta^{18}O_C$)	T°C med ($\delta^{18}O_P$)	T°C med ($\delta^{18}O_C$)	T°C CAO ($\delta^{18}O_P$)	T°C CAO ($\delta^{18}O_C$)	$\delta^{18}O_W$VMSOW (18°)	$\delta^{18}O_W$VMSOW (25°)	$\delta^{18}O_W$VMSOW (32°)
F2	23.28	22.37	33.29	34.01	32.21	32.69	28.62	28.41	-1.83	-0.21	1.42
F3	24.70	23.89	34.71	35.72	33.63	34.38	30.04	30.03	-2.16	-0.54	1.09
F6	24.14	23.27	34.15	35.02	33.07	33.69	29.48	29.37	-2.03	-0.41	1.22
F9	25.73	25.04	35.74	37.02	34.66	35.66	31.08	31.26	-2.40	-0.78	0.85
F10	25.47	24.75	35.48	36.69	34.41	35.34	30.82	30.95	-2.34	-0.72	0.91
F11	29.81	29.60	39.83	42.13	38.75	40.72	35.16	36.12	-3.35	-1.73	-0.10
F12	30.07	29.86	40.08	42.42	39.01	41.00	35.42	36.39	-3.41	-1.79	-0.16
F14	29.30	29.00	39.31	41.45	38.23	40.05	34.64	35.47	-3.23	-1.61	0.02
F15	26.33	25.67	36.34	37.73	35.27	36.36	31.68	31.93	-2.54	-0.92	0.71
F16	32.35	32.54	42.36	45.42	41.29	43.96	37.70	39.24	-3.94	-2.32	-0.69
F17	32.31	32.49	42.32	45.36	41.24	43.91	37.65	39.19	-3.93	-2.31	-0.68
F18	25.77	25.09	35.78	37.07	34.71	35.71	31.12	31.31	-2.41	-0.79	0.84
F19	35.06	35.78	45.07	49.02	44.00	47.53	40.41	42.68	-4.57	-2.95	-1.32
F20	30.89	30.88	40.90	43.56	39.82	42.13	36.24	37.48	-3.60	-1.98	-0.35
T26	29.12	28.84	39.14	41.29	38.06	39.88	34.47	35.31	-3.19	-1.57	0.06
T28	23.06	22.14	33.08	33.74	32.00	32.43	28.41	28.16	-1.78	-0.16	1.47
T29	23.15	22.23	33.16	33.85	32.08	32.53	28.50	28.26	-1.80	-0.18	1.45
T30	25.34	24.61	35.35	36.53	34.28	35.18	30.69	30.80	-2.31	-0.69	0.94
T31	22.33	21.39	32.34	32.90	31.27	31.59	27.68	27.36	-1.61	0.01	1.64
T32	30.41	30.27	40.43	42.88	39.35	41.45	35.76	36.83	-3.49	-1.87	-0.24
T34	26.54	25.92	36.56	38.00	35.48	36.63	31.89	32.19	-2.59	-0.97	0.66
T35	19.54	18.52	29.55	29.65	28.47	28.38	24.88	24.29	-0.96	0.66	2.29
T36	22.33	21.39	32.34	32.90	31.27	31.59	27.68	27.36	-1.61	0.01	1.64
T37	29.55	29.30	39.57	41.79	38.49	40.38	34.90	35.80	-3.29	-1.67	-0.04
P59	29.64	29.35	39.65	41.85	38.58	40.44	34.99	35.85	-3.31	-1.69	-0.06
Pi63	25.43	24.70	35.44	36.64	34.36	35.28	30.77	30.90	-2.33	-0.71	0.92

C69	26.80	26.21	36.82	38.33	35.74	36.96	32.15	32.51	-2.65	-1.03	0.60
C70	28.14	27.69	38.15	39.99	37.07	38.60	33.48	34.09	-2.96	-1.34	0.29
C71	26.85	26.26	36.86	38.38	35.78	37.01	32.19	32.56	-2.66	-1.04	0.59
C72	29.68	29.45	39.70	41.96	38.62	40.55	35.03	35.96	-3.32	-1.70	-0.07
C73	29.30	29.00	39.31	41.45	38.23	40.05	34.64	35.47	-3.23	-1.61	0.02
C74	29.12	28.79	39.14	41.23	38.06	39.82	34.47	35.26	-3.19	-1.57	0.06
C75	24.48	23.70	34.49	35.50	33.42	34.16	29.83	29.83	-2.11	-0.49	1.14
C76	28.39	27.99	38.41	40.33	37.33	38.93	33.74	34.41	-3.02	-1.40	0.23
Picci84	27.66	27.15	37.68	39.38	36.60	38.00	33.01	33.51	-2.85	-1.23	0.40

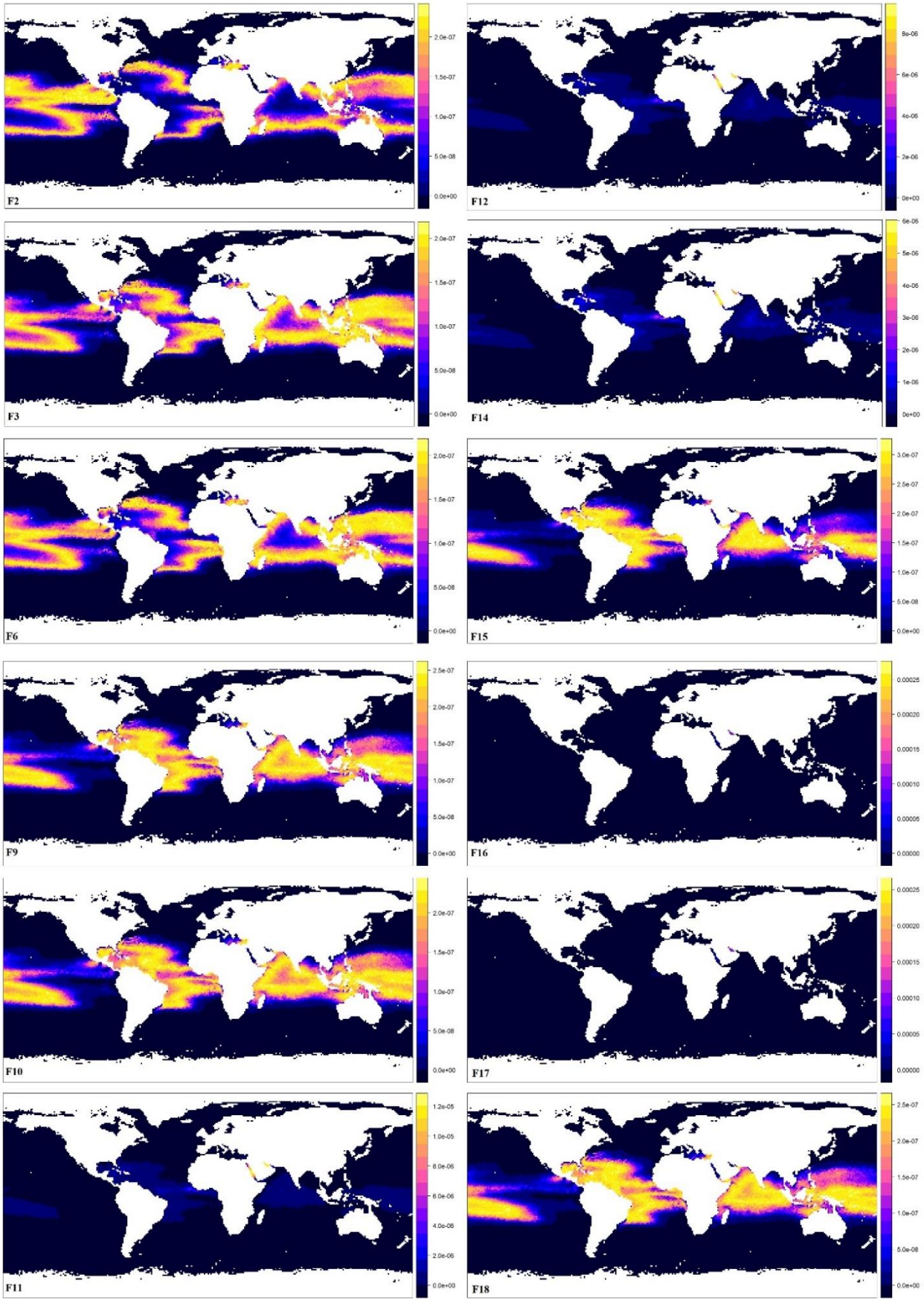

Figure S1 Estimated probability of the 35 sawfish teeth origin calculated from the values of the oxygen isotopes. Yellow colours showed the highest probability and dark blue the lowest.

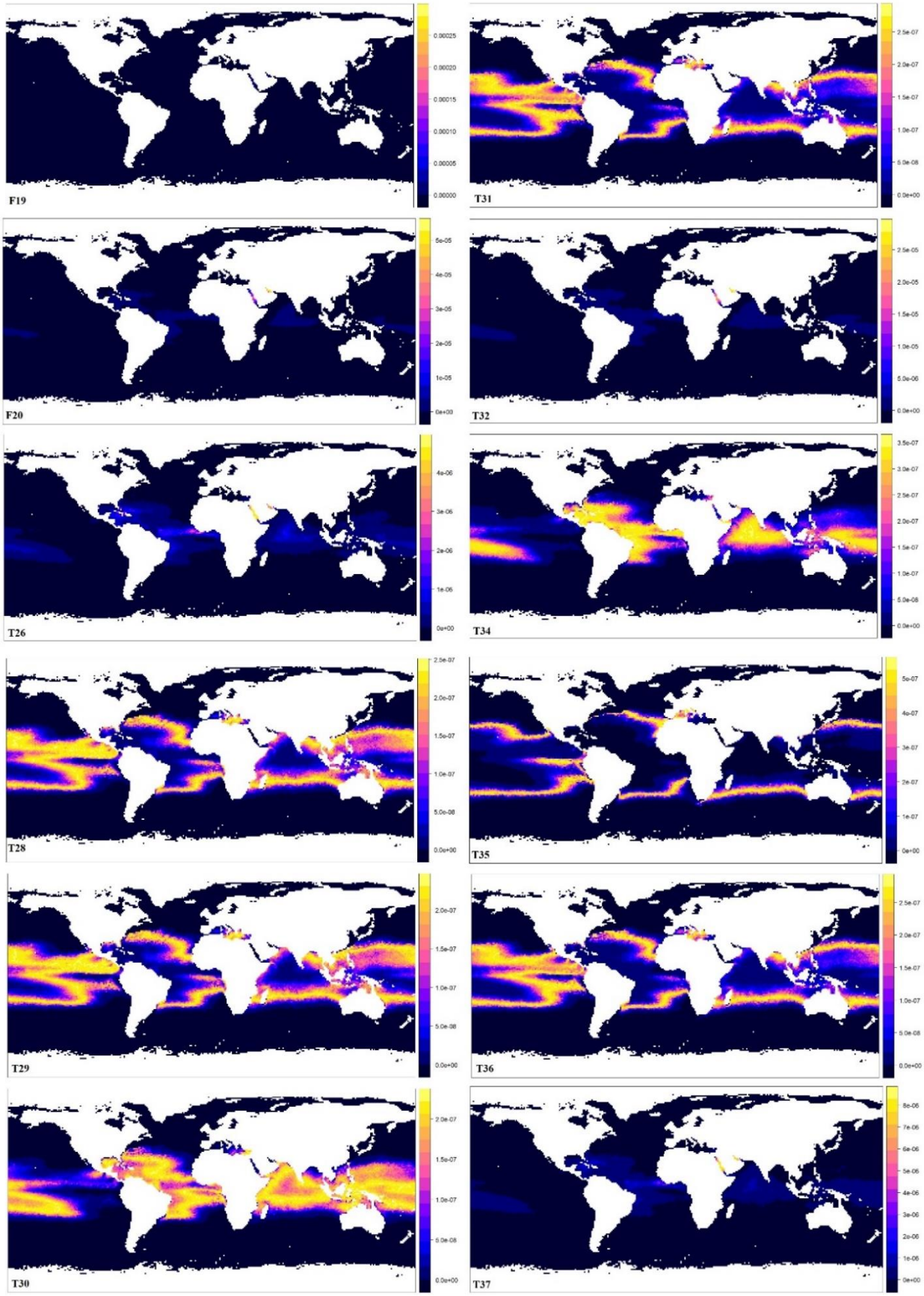

Figure S1 Estimated probability of the 35 sawfish teeth origin calculated from the values of the oxygen isotopes. Yellow colours showed the highest probability and dark blue the lowest.

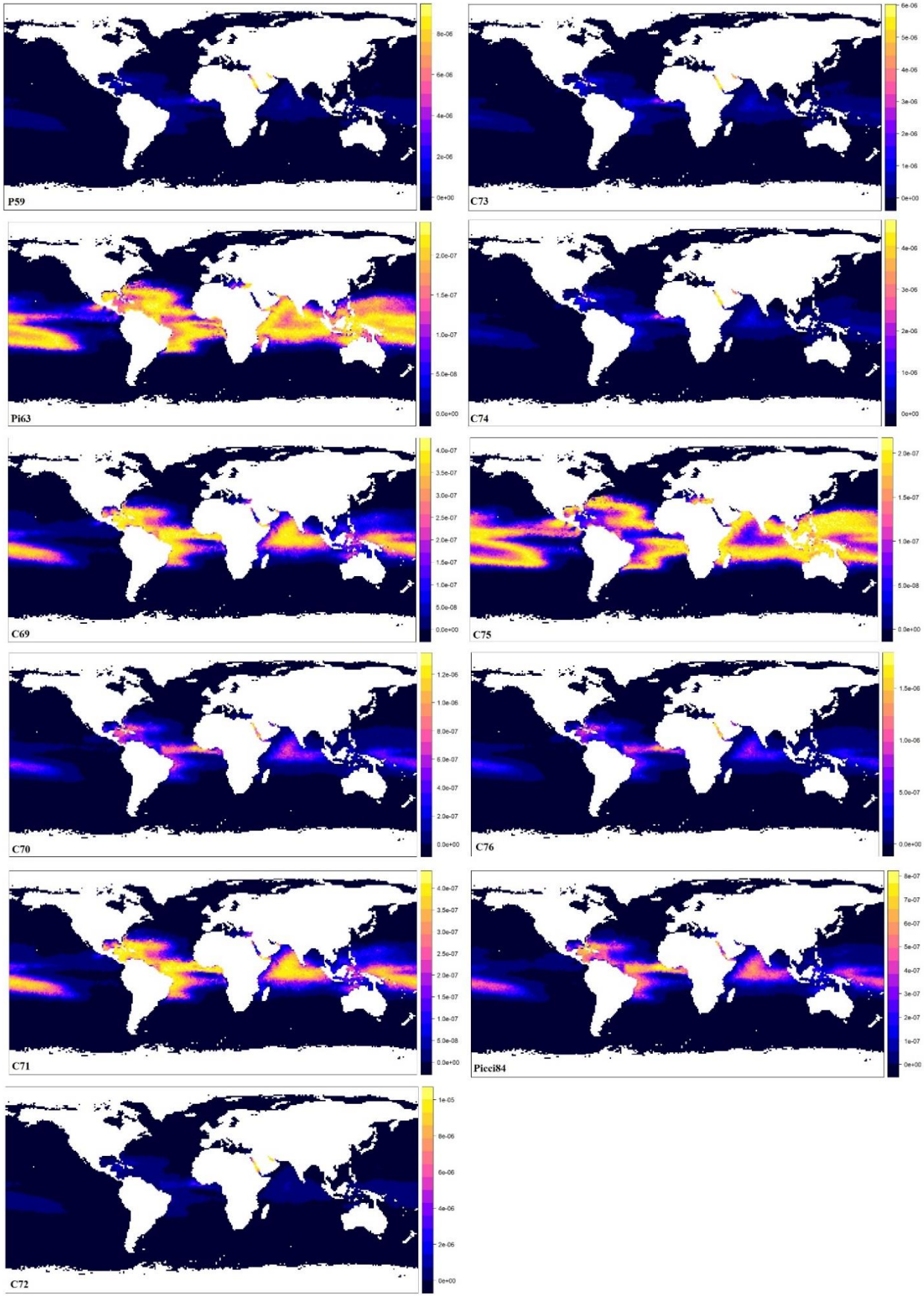

Figure S1 Estimated probability of the 35 sawfish teeth origin calculated from the values of the oxygen isotopes. Yellow colours showed the highest probability and dark blue the lowest.

Chapter 4

Preliminary results of microstructural and strontium isotope analysis of rostral teeth of Pristis zijsron: potential for ageing and assessing habitat references and movements of historical sawfishes

4.1 Introduction

Assessing the ecology at appropriate spatial and temporal scales of highly endangered species can be considered a challenge due to their low abundance. However, understanding the distribution and ecology of sawfish, especially in their remaining stronghold regions, is necessary to define conservation actions beyond the implementation of legal limits on catch and trade in sawfish parts (Dulvy et al., 2016). All species of sawfish move across a range of seawater salinities from salty to brackish environments (Poulakis et al., 2011; Scharer et al., 2012; Faria et al., 2013). The behaviour of sawfish populations in relation to salinity is well studied in north Australia only for the largetooth sawfish *Pristis pristis* L. (Peverell, 2005; Whitty et al., 2009; Gleiss et al., 2017) and in the United States for the smalltooth sawfish *P. pectinata* Latham, 1794 by direct studies of movements and locations using telemetry (Norton et al., 2012; Graham et al., 2021).

Museum and private archives provide a reservoir of samples from which to uncover details of sawfish life history without harming living individuals (Phillips et al., 2009; Whitty et al., 2014). Increasing the data richness of historical samples allows the reconstruction of the historical ecology

and past distribution, and potentially reflect species life history before and during the ongoing declines in abundance (Whitty et al., 2014).

Isotopic and trace elemental analyses of fish hard parts are particularly powerful for reconstructing habitat preferences, birth place, movements, and other life-history traits (Hobson, Barnett-Johnson, & Cerling, 2010; Tillett et al., 2011; Smith, Miller, & Heppell, 2013). As a matter of fact, some elements readily substitute for calcium and are incorporated in the crystalline of calcareous structures which grow throughout the entire life of the fish (Elsdon et al., 2008). Strontium (Sr) isotope composition, $^{87}Sr/^{86}Sr$, can vary greatly in nature and has been used to trace fish movements from waters with different salinity as freshwater, marine, and brackish habitats (Kocsis, Vennemann, & Fontignie, 2007; Scharer et al., 2012; Fischer et al., 2013; Benito et al., 2021; Hegg, Graves, & Fisher, 2021; Wong et al., 2021; Zieliński et al., 2021).

Rostral teeth of sawfish are highly modified dermal denticles which grow through the life of the individual (Welten et al., 2015) and are composed of a complex matrix of hydroxyapatite (Miller, 1974). Apatite structures could replace Calcium (Ca) with Sr cations (Blum et al., 2000; Vennemann et al., 2001). As a result, sawfish rostral teeth may provide a useful record of age, growth and environmental chemistry which could hold information on their life history and movements (Field, Meekan, & Bradshaw, 2009; Hegg, Graves, & Fisher, 2021).

In this chapter I present preliminary data from the analysis of rostral teeth taken from sawfish dried rostra of two *Pristis zijsron* from the Red Sea. The main purpose is to assess the presence of growth structures in the teeth that could be used to estimate the individual age and growth information. In addition, I preliminarily estimated the rostral tooth isotopic composition of the strontium ($^{87}Sr/^{86}Sr$) using laser ablation–inductively coupled plasma mass spectrometry (LA-ICPMS) to assess the movement patterns and habitat use occurring during the life of individual sawfish, and specifically whether they move across salinity gradients.

4.2 Materials and methods

4.2.1 Sample information

Two rostral teeth of *P. zijsron* individuals (F20 and Picci83) likely originated from Persian Gulf and Red Sea (see Chapters 2 and 3) were selected to test the sectioning and reading procedures for microstructural analysis of thin sections. Since the methodological purpose of this task, these rostral teeth were selected for this destructive technique because they appeared heavily damaged and of scarce value for exposition in the museum.

The sample F20 (museum label: 572) belonged to the collection of the Natural History Museum "La Specola" of the University of Firenze (Figure 1) and included the rostrum and first part of the cranium. The rostrum appeared ruined and with many lost teeth. The total rostrum length measured 1450 mm and the standard rostrum length 1270 mm corresponding to a large adult with an estimated total length of 4247.09 mm (see Table S4 of the Chapter 2). No documental geographical origin has been recovered from the Museum (Vanni, 1992), but thanks to the analysis of Oxygen and Carbon isotopes conducted to a different tooth of the same rostrum, the individual was precisely assigned to the Persian Gulf (Figure 9 and Table 2 of Chapter 3).

The analysed tooth was taken from the most distal part of the rostrum and measured 63 mm of longitudinal length and 11 mm of width at the base.

Figure 1 Rostrum (F20) conserved in the Natural History Museum "La Specola", University of Firenze. The adult *P. zijsron* had unknown documental origin but was precisely assigned to the Persian Gulf by previous analyses of this thesis. Total rostral length of 1450 mm and a standard rostral length of 1270 mm. The most distal tooth on the left-hand side was taken for analysis.

The second tooth analysed belonged to one of the three private Piccinetti archive rostra located at Fano (PU, Italy) (Figure 2). The total rostrum length measured 744 mm and the standard rostrum length 660 mm corresponding to a young individual with an estimated total length of 2425.39 mm (see Table S4 of the Chapter 2). The documental geographical origin has been recovered as Red Sea origin (C. Piccinetti personal communication). The tooth was not included in the Oxygen and Carbon isotope analysis, but a second tooth of the collection, Picci84, with the same sampling information and condition was assigned to the Red Sea (Figure 5 and Table 2 of Chapter 3). The analysed tooth was the most proximal of the rostrum from the right-hand side and measured 13 mm of longitudinal length and 5 mm of width at the base.

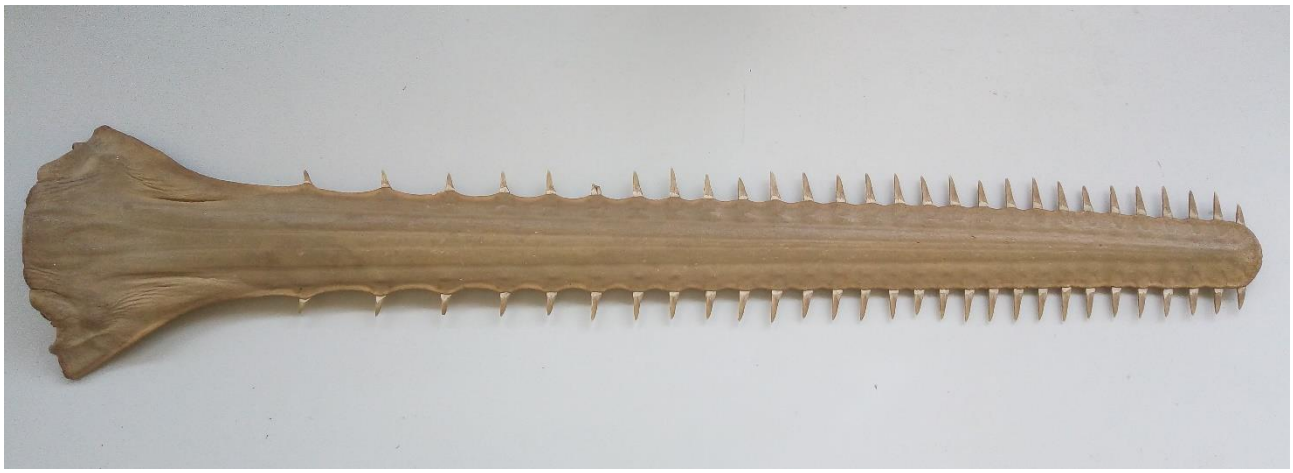

Figure 2 Rostrum (Picci83) from the private Piccinetti collection located at Fano (PU, Italy). The rostrum was collected in Etiopia, Red Sea in 1939-40 and had a total rostrum length of 744 mm and a standard rostrum length of 660 mm. The most proximal tooth on the right-hand side was taken for analysis.

4.2.2 Sectioning and imaging of rostral teeth

To perform the histological examination of the rostral teeth, we followed the procedure described in Nava et al. (2020). Before sectioning, each tooth was cleaned by sonication in an ultrasonic cleaner. After cleaning, the rostral teeth were left to dry and then embedded in epoxy resin (EpoThin 2; Buehler) and cured for 48 h at room temperature (Nava et al., 2020). The embedding is necessary to provide stability to the sample during the subsequent cutting procedure.

The sectioning was performed with an IsoMet low-speed saw (Buehler) mounted with a diamond blade. The first cut, passing by the tip of the tooth, was performed along the dorsal-ventral plane of the teeth. The resulting halves of each tooth was then attached to a microscope glass slide, previously treated with ethanol 95% and then with ethanol 100%, by using a thin layer of epoxy resin. At this point, a second cut was performed parallel to the glass slide and a thin section of 250 µm of thickness was obtained. Water-resistant abrasive paper of different grits (Carbimet; Buehler) was then used to grind the section to approximately 150 µm.

Finally, each section was polished on a polishing cloth (Buehler) by using 1 μm polycrystalline diamond suspension (Buehler) (Nava et al., 2020). The image of each thin section was recorded using an Axioscope 7 metallographic microscope with Axiocam 208 colour camera (Zeiss, Oberkochen, Germany) at 10x magnification for Picci83 and 5x magnification for F20. The assembly of the micrographs was performed by using the mosaic function of ZenCore 3 software (Zeiss, Oberkochen, Germany). Each image was subsequently checked for any cross striation and Retzius lines (growth lines seen in tooth enamel) (Nava et al., 2020) or other sequential growth structures.

4.2.3 Strontium isotope composition

The two thin sections were tested for the Sr isotopic composition at the Department of Chemical and Geological Sciences of the University of Modena and Reggio-Emilia, using laser ablation MC-ICPMS (Multicollector-Inductively Coupled Plasma Mass Spectrometer) to possibly detect habitat shifts of the animals. Before starting the analysis, each tooth was cleaned with MilliQ water, accurately dried and the surface was pre–ablated. The $^{87}Sr/^{86}Sr$ composition was measured by a Neptune MC-ICPMS coupled to a 213 nm Nd:YAG laser ablation system (New Wave Research ^{TM}).

To correct Krypton (Kr) interferences the "on peak zero" method was used. This consists in measuring gas background for 60 s for each mass before each analysis and subtracting the background signal of each detector to the measured sample value. After background subtraction, the remaining signal of mass 82, was used to check the formation of Ca, isobars to masses ^{84}Sr, ^{86}Sr, ^{87}Sr, and ^{88}Sr. Mass 85 was used to correct the signal on mass 87 for the presence of isobaric Rubidium (Rb), using an $^{87}Rb/^{85}Rb$ ratio of 0.3856656. Mass bias normalization, through exponential law, was carried out using an $^{88}Sr/^{86}Sr$ ratio of 8.375209 (Lugli et al., 2017).

The Neptune MC-ICPMS was tuned using an in-house bioapatite reference material (i.e., a modern shark tooth, ST1) monitoring both the signal and the isotopic ratios. Specifically, the daily LA–MC–ICPMS tuning is based on a 3-steps protocol. During the first step, ST1 house-reference material was analysed to optimize the machine parameters tuning for the maximum signal intensity on ^{88}Sr. Second, background signal intensities on ^{83}Kr and ^{82}Kr are monitored. At the end, the $^{87}Sr/^{86}Sr$ ratio of the ST1 standard was measured and the machine parameters were acquired with a block of 200 cycles, an integration time of 0.5 s, turned until the desired ratio of ~0.7092 (Lugli et al., 2017) was obtained. A daily calibration curve was then constructed using three teeth as standard, namely a bovine tooth, swine tooth and shark tooth (Lugli et al., 2017), to correct polyatomic interferences on mass 87 (i.e., Ar-CaPO). Finally, for each tooth, we obtained an $^{87}Sr/^{86}Sr$ profile for the entire tooth length, in our case from the tip to the cervix (from juvenile to adult life stages).

4.3 Results and discussion

4.3.1 Structural analysis of rostral teeth

Composite images of the thin sections are made onto two test tooth samples (Figure 1 and Figure 2). The rostral teeth seemed to contain a mesh-like network of interconnected tubules as described by Miller (1974), but it was too indistinct to be easily quantified. Unfortunately, no clear evidence of daily or annual growth of microstructures was obtained, neither Retzius lines nor any other isochronous growth structure that could be used to build an accurate temporal sequence within the tooth were visible. It was only possible to sense the directionality of the growth. The lack of growth microstructures was probably due to the heavy worn of both teeth. Other thin sections could be processed, but there is no certainty of seeing any growth structure. The very preliminary attempt performed to age these individuals wants to make up for the lack of information about any cross striation, Retzius lines, or other sequential growth structures possibly present in sawfish rostral teeth. These microstructures are usually present in the tooth enamel (Nava et al., 2020), the external layer easily ruined by the repeated use of the saw for feeding (Wueringer, 2012; Nevatte et al., 2017). Analysis of banding structure and age lines are well studied with remarkable results, using otolith for fish (Xieu et al., 2021) or using tooth enamel for human populations (Nava et al., 2020). Hegg, Graves, & Fisher (2021) found putative growth bands with a linear distance relationship in a rostral tooth of a sub-adult

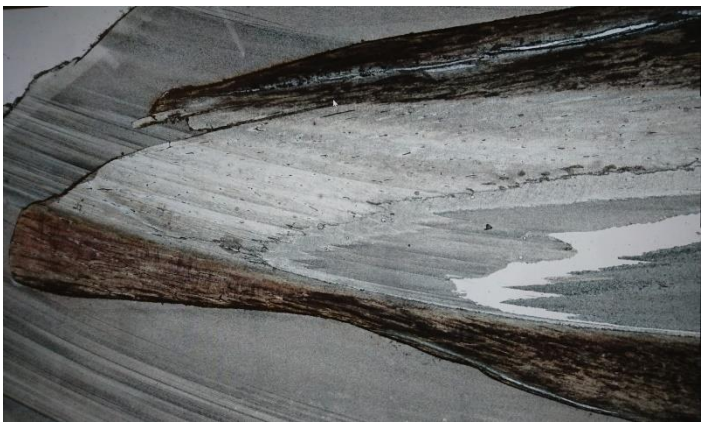

Figure 1. Thin section performed on a tooth of *P. zijsron* from the Museum of Florence (F20), sectioned on the dorsal-ventral plane. A network of interconnected tubules was weakly visible for a proper quantification of banding structures

Figure 2. Thin section performed on a tooth of *P. zijsron* from the Piccinetti private collection (Picci83), sectioned on the dorsal-ventral plane. A network of interconnected tubules was weakly visible for a proper quantification of banding structures.

largetooth sawfish. They performed different process, such as unstained, with silver nitrate staining, burning and Mutvei's solution. Only the section stained with Mutvei's solution provided the image for measurement for the quantification of the apparent banding.

Further staining processes must be perform and test in the two teeth analysed in this study to better determine the quantification of banding structures related to the grow and the age of the animals.

4.3.2 Sr isotopic composition

The Sr isotopic composition outlines (Figure 3 and Figure 4) of rostral teeth ranged closely near the $^{87}Sr/^{86}Sr$ isotopic composition = 0.7092, which perfectly match modern seawater reference value (i.e., 0.70918) from the cervix to tip of teeth. The constant $^{87}Sr/^{86}Sr$ ratio outline on the rostral teeth from juvenile to adult stages around the modern seawater value across the entire individual life, indicated that these two individuals of *P. zijsron* spent all their life in marine habitats. This could be expected considering that *P. zijsron* is less tolerant to low-saline conditions than *P. pristis* and *P. pectinata*, and it remains close to the mouth of the river even in early life (Morgan et al., 2017).

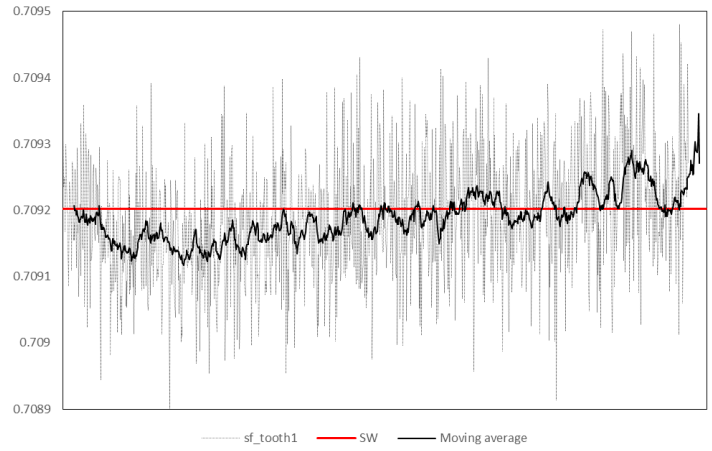

Figure 3. Outline of $^{87}Sr/^{86}Sr$ isotopic composition of *P. zijsron* tooth sample F20.

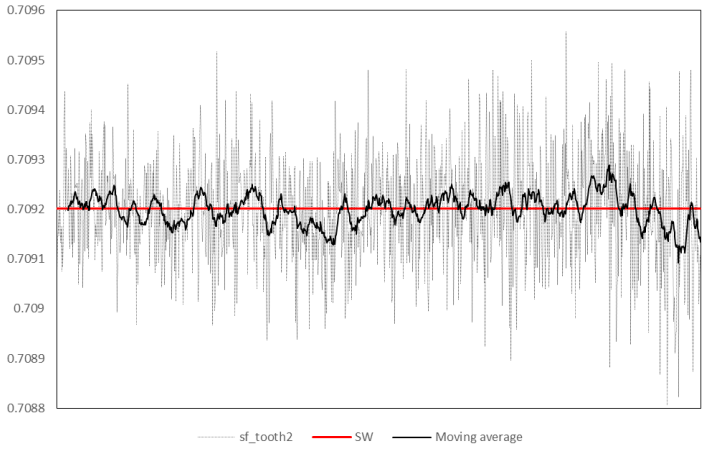

Figure 4. Outline of $^{87}Sr/^{86}Sr$ isotopic composition of tooth *P. zijsron* tooth sample Picci83.

Strontium isotope composition is well mixed in ocean water with a signature of 0.70918 (Faure & Mensing, 2005). Deviations from this ratio indicate movement into fresh or brackish water. Hegg, Graves, & Fisher (2021) demonstrated how powerful is this tool applied in sawfish rostral teeth, which are available recorder of chemical information throughout the life of individuals.

Samples of *Pristis pristis* used in the study highlighted movement in freshwater during early life, followed by the use of multiple salinities later in life. It is known that juveniles of largetooth sawfish usually utilize freshwater habitats as nursery areas (Wueringer, Squire, & Collin, 2009).

Future analysis of Sr outline could be processed considering more individuals, to test the difference that occurs between species and to evaluate their movements across salinity. Moreover, the analyses of the isotopic composition of teeth could be improved with the oxygen and carbon analysis to assess the correct past distribution of sawfish species. Combining with isoscape techniques and other spatial data made possible to uncover important habitat and behaviour (Hobson, Barnett-Johnson, & Cerling, 2010; Hamann et al., 2014; Smith et al., 2016; Brennan & Schindler, 2017). Finally, combining chemical information with ongoing genetic analyses using archived rostra could also provide greater detail in population connectivity and movement (Fearing et al., 2018).

The development of methods which can clarify life history and movement patterns of highly endangered sawfish species is required to understand their ecology and improve conservation efforts. Current knowledge of the habitat use of sawfish populations is limited in many areas. Provenance and life history can be investigated using rostral tooth chemistry (Walther, 2019; Wang, Walther, & Gillanders, 2019). The results must necessarily be considered as preliminary given the poor number of samples analysed here. Larger samples sizes could confirm our findings, including sawfish of known origin which can be compared with known water chemistries. The large numbers of rostra conserved in museums, academic institutions and private collections around the World could be employed to improve the knowledge of sawfish ecology which are difficult to study in extant populations.

4.4 References

Benito, M. I., Suarez-Gonzalez, P., Quijada, I. E., Campos-Soto, S., & Rodríguez-Martínez, M. (2021). Constraints of applying strontium isotope stratigraphy in coastal and shallow marine environments: insights from Lower Cretaceous carbonates deposited in an active tectonic setting (N Iberian Basin, Spain). *J. Iber. Geol.***47,** 151–169.

Blum, J. D., Taliaferro, E. H., Weisse, M. T., & Holmes, R. T. (2000). Changes in Sr/Ca, Ba/Ca and 87Sr/86Sr ratios between trophic levels in two forest ecosystems in the northeastern U.S.A. *Biogeochemistry* **49**, 87–101.

Brennan, S. R., & Schindler, D. E. (2017). Linking otolith microchemistry and dendritic isoscapes to map heterogeneous production of fish across river basins. *Ecol. Appl.* **27**, 363–377.

Dulvy, N. K., Davidson, L. N. K., Kyne, P. M., Simpfendorfer, C. A., Harrison, L. R., Carlson, J. K., & Fordham, S. V. (2016). Ghosts of the coast: global extinction risk and conservation of sawfishes. *Aquat. Conserv. Mar. Freshw. Ecosyst.* **26**, 134–153.

Elsdon, T., Wells, B., Campana, S., Gillanders, B., Jones, C., Limburg, K., Secor, D., Thorrold, S., & Walther, B. (2008). Otolith Chemistry To Describe Movements And Life-History Parameters Of Fishes. In *Oceanogr. Mar. Biol. Annu. Rev.* Vol. 46, pp. 297–330.

Faria, V. V., McDavitt, M. T., Charvet, P., Wiley, T. R., Simpfendorfer, C. A., & Naylor, G. J. P. (2013). Species delineation and global population structure of Critically Endangered sawfishes (Pristidae). *Zool. J. Linn. Soc.* **167**, 136–164.

Faure, G., & Mensing, T. M. (2005). *Isotopes: Principles and Applications.* Hoboken, N.J.: Wiley.

Fearing, A., Smith, K., Wiley, T., Whitty, J., Feldheim, K., Kyne, P., & Phillips, N. (2018). Looking Back for the Future: Utilizing Sawfish Saws from Natural History Collections to Conserve the Critically Endangered Largetooth Sawfish (Pristis pristis). *Biodivers. Inf. Sci. Stand.* **2**, e25806.

Field I.C., Meekan M.G. & Bradshaw C.J.A. (2009). Development of non-lethal methods for determining age and habitat use of sawfishes from northern Australia. Final Report.

Fischer, J., Schneider, J. W., Voigt, S., Joachimski, M. M., Tichomirowa, M., Tütken, T., Götze, J., & Berner, U. (2013). Oxygen and strontium isotopes from fossil shark teeth: Environmental and ecological implications for Late Palaeozoic European basins. *Chem. Geol.* **342**, 44–62.

Gleiss, A. C., Morgan, D. L., Whitty, J. M., Keleher, J. J., Fossette, S., & Hays, G. C. (2017). Are vertical migrations driven by circadian behaviour? Decoupling of activity and depth use in a large riverine elasmobranch, the freshwater sawfish (Pristis pristis). *Hydrobiologia* **787**, 181–191.

Graham, J., Kroetz, A. M., Poulakis, G. R., Scharer, R. M., Carlson, J. K., Lowerre-Barbieri, S., Morley, D., Reyier, E. A., & Grubbs, R. D. (2021). Large-scale space use of large juvenile and adult smalltooth sawfish Pristis pectinata: implications for management. *Endanger. Species Res.* **44**, 45–59.

Hamann, E. J., Kennedy, B. P., Whited, D. C., & Stanford, J. A. (2014). Spatial Variability in Spawning Habitat Selection by Chinook Salmon (oncorhynchus Tshawytscha) in a Wilderness River. *River Res. Appl.* **30**, 1099–1109.

Hegg, J. C., Graves, B., & Fisher, C. M. (2021). Sawfish, read in tooth and saw: Rostral teeth as endogenous chemical records of movement and life-history in a critically endangered species. *Aquat. Conserv. Mar. Freshw. Ecosyst.* **31**(9), 2334–2347.

Hobson, K. A., Barnett-Johnson, R., & Cerling, T. (2010). Using Isoscapes to Track Animal Migration. In J. B. West, G. J. Bowen, T. E. Dawson, & K. P. Tu (Eds.), *Isoscapes Underst. Mov. Pattern Process Earth Isot. Mapp.* pp. 273–298. Dordrecht: Springer Netherlands.

Kocsis, L., Vennemann, T. W., & Fontignie, D. (2007). Migration of sharks into freshwater systems during the Miocene and implications for Alpine paleoelevation. *Geology* **35**, 451–454.

Lugli, F., Cipriani, A., Peretto, C., Mazzucchelli, M., & Brunelli, D. (2017). In situ high spatial resolution 87Sr/86Sr ratio determination of two Middle Pleistocene (c.a. 580 ka) Stephanorhinus hundsheimensis teeth by LA–MC–ICP–MS. *Int. J. Mass Spectrom.* **412**, 38–48.

Miller, W. A. (1974). Observations on the Developing Rostrum and Rostral Teeth of Sawfish: Pristis perotteti and P. cuspidatus. *Copeia* **1974**, 311–318.

Morgan, D. L., Ebner, B. C., Allen, M. G., Gleiss, A. C., Beatty, S. J., & Whitty, J. M. (2017). Habitat use and site fidelity of neonate and juvenile green sawfish Pristis zijsron in a nursery area in Western Australia. *Endanger. Species Res.* **34**, 235–249.

Nava, A., Lugli, F., Romandini, M., Badino, F., Evans, D., Helbling, A. H., Oxilia, G., Arrighi, S., Bortolini, E., Delpiano, D., Duches, R., Figus, C., Livraghi, A., Marciani, G., Silvestrini, S., Cipriani, A., Giovanardi, T., Pini, R., Tuniz, C., Bernardini, F., Dori, I., Coppa, A., Cristiani, E., Dean, C., Bondioli, L., Peresani, M., Müller, W., & Benazzi, S. (2020). Early life of Neanderthals. *Proc. Natl. Acad. Sci. U. S. A.* **117**, 28719–28726.

Nevatte, R. J., Wueringer, B. E., Jacob, D. E., Park, J. M., & Williamson, J. E. (2017). First insights into the function of the sawshark rostrum through examination of rostral tooth microwear. *J. Fish Biol.* **91**, 1582–1602.

Norton, S. L., Wiley, T. R., Carlson, J. K., Frick, A. L., Poulakis, G. R., & Simpfendorfer, C. A. (2012). Designating Critical Habitat for Juvenile Endangered Smalltooth Sawfish in the United States. *Mar. Coast. Fish.* **4**, 473–480.

Parris, K. M., McCall, S. C., McCarthy, M. A., Minteer, B. A., Steele, K., Bekessy, S., & Medvecky, F. (2010). Assessing ethical trade-offs in ecological field studies. *J. Appl. Ecol.* **47**, 227–234.

Peverell, S. C. (2005). Distribution of sawfishes (Pristidae) in the Queensland Gulf of Carpentaria, Australia, with notes on sawfish ecology. *Environ. Biol. Fishes* **73**, 391–402.

Phillips, N., Chaplin, J., Morgan, D., & Peverell, S. (2009). Extraction and amplification of DNA from the dried rostra of sawfishes (Pristidae) for applications in conservation genetics. *Pac. Conserv. Biol.* **15**, 128–134.

Poulakis, G. R., Stevens, P. W., Timmers, A. A., Wiley, T. R., Simpfendorfer, C. A., Poulakis, G. R., Stevens, P. W., Timmers, A. A., Wiley, T. R., & Simpfendorfer, C. A. (2011). Abiotic affinities and spatiotemporal distribution of the endangered smalltooth sawfish, Pristis pectinata, in a south-western Florida nursery. *Mar. Freshw. Res.* **62**, 1165–1177.

Scharer, R. M., Patterson, W. F., Carlson, J. K., & Poulakis, G. R. (2012). Age and Growth of Endangered Smalltooth Sawfish (Pristis pectinata) Verified with LA-ICP-MS Analysis of Vertebrae. *PLoS ONE* **7**, 1–8.

Smith, W. D., Miller, J. A., & Heppell, S. S. (2013). Elemental Markers in Elasmobranchs: Effects of Environmental History and Growth on Vertebral Chemistry. *PLOS ONE* **8**, e62423.

Smith, W. D., Miller, J. A., Márquez-Farías, J. F., & Heppell, S. S. (2016). Elemental signatures reveal the geographic origins of a highly migratory shark: prospects for measuring population connectivity. *Mar. Ecol. Prog. Ser.* **556**, 173–193.

Tillett, B. J., Meekan, M. G., Parry, D., Munksgaard, N., Field, I. C., Thorburn, D., & Bradshaw, C. J. A. (2011). Decoding fingerprints: elemental composition of vertebrae correlates to age-related habitat use in two morphologically similar sharks. *Mar. Ecol. Prog. Ser.* **434**, 133–142.

Vennemann, T. W., Hegner, E., Cliff, G., & Benz, G. W. (2001). Isotopic composition of recent shark teeth as a proxy for environmental conditions. *Geochim. Cosmochim. Acta* **65**, 1583–1599.

Walther, B. D. (2019). The art of otolith chemistry: interpreting patterns by integrating perspectives. *Mar. Freshw. Res.* **70**, 1643–1658.

Wang, C.-H., Walther, B. D., & Gillanders, B. M. (2019). Introduction to the 6th International Otolith Symposium. *Mar. Freshw. Res.* **70**, i–iii.

Welten, M., Smith, M. M., Underwood, C., & Johanson, Z. (2015). Evolutionary origins and development of saw-teeth on the sawfish and sawshark rostrum (Elasmobranchii; Chondrichthyes). *R. Soc. Open Sci.* **2**, 150189.

Whitty, J. M., Morgan, D. L., Peverell, S. C., Thorburn, D. C., Beatty, S. J., Whitty, J. M., Morgan, D. L., Peverell, S. C., Thorburn, D. C., & Beatty, S. J. (2009). Ontogenetic depth partitioning by juvenile freshwater sawfish (Pristis microdon: Pristidae) in a riverine environment. *Mar. Freshw. Res.* **60**, 306–316.

Whitty, J. M., Phillips, N. M., Thorburn, D. C., Simpfendorfer, C. A., Field, I., Peverell, S. C., & Morgan, D. L. (2014). Utility of rostra in the identification of Australian sawfishes (Chondrichthyes: Pristidae). *Aquat. Conserv. Mar. Freshw. Ecosyst.* **24**, 791–804.

Wong, M., Grimes, V., Steskal, M., Song, J., Ng, J., Jaouen, K., Lam, V. C., & Richards, M. (2021). A bioavailable baseline strontium isotope map of southwestern Turkey for mobility studies. *J. Archaeol. Sci. Rep.* **37**, 102922.

Wueringer, B. E. (2012). Electroreception in Elasmobranchs: Sawfish as a Case Study. *Brain. Behav. Evol.* **80**, 97–107.

Wueringer, B. E., Squire, L., & Collin, S. P. (2009). The biology of extinct and extant sawfish (Batoidea: Sclerorhynchidae and Pristidae). *Rev. Fish Biol. Fish.* **19**, 445.

Xieu, W., Lewis, L. S., Zhao, F., Fichman, R. A., Willmes, M., Hung, T. C., Ellison, L., Stevenson, T., Tigan, G., Schultz, A. A., & Hobbs, J. A. (2021). Experimental validation of otolithbased age and growth reconstructions across multiple life stages of a critically endangered estuarine fish. *PeerJ* **9**, 1–22.

Zieliński, M., Dopieralska, J., Królikowska-Ciągło, S., Walczak, A., & Belka, Z. (2021). Mapping of spatial variations in Sr isotope signatures (87Sr/86Sr) in Poland — Implications of anthropogenic Sr contamination for archaeological provenance and migration research. *Sci. Total Environ.* **775**, 145792.