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OXFORD

Original Article

Revisiting historical diversity of sawfishes (Pristidae) in the Mediterranean Sea using natural history collections

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ABSTRACT

Sawfishes are highly threatened elasmobranchs, now locally extinct in many coastal areas worldwide. Global conservation efforts emphasize documenting historical shifts in species' distribution and range. In the Mediterranean Sea, these historical reconstructions suggested the past presence of two species, *Pristis pristis* and *Pristis pectinata*, thought extinct by the 1970s, revising prior assumptions on sawfish distribution. In this study, we evaluated whether any historical sawfish specimens housed in several European institutions and private collections might represent records from the Mediterranean Sea. We analysed 229 rostra, of which 28 were labelled as Mediterranean individuals. Most specimens lacked detailed taxonomic identification or reliable collection data. Using morphometrics and mitochondrial gene sequencing (partial *COI* and *NADH2*) we identified all rostra as belonging to four extant sawfish species: *Pristis zijsron* (104), *Anoxypristis cuspidata* (52), *P. pristis* (47), and *P. pectinata* (26). Specimens labelled as Mediterranean belonged to *P. zijsron* (9), *A. cuspidata* (8), *P. pristis* (6), and *P. pectinata* (5). These findings demonstrate the effectiveness of an integrative framework combining molecular and morphometric analyses for reliable species' identification in historical sawfish specimens, providing a robust basis for re-evaluating historical diversity and biogeography even when collection metadata are incomplete or uncertain.

Keywords: historical DNA; elasmobranchs; morphometrics; museomics; natural history collections

INTRODUCTION

Sharks, rays, and chimaeras are highly threatened by human impacts, such as fishing and habitat degradation (Dulvy *et al.* 2021). Declines in population abundance and reduction of home range have led many species to the brink of extinction (Dulvy *et al.* 2014). Sawfishes (family Pristidae) are among the most threatened elasmobranchs (Dulvy *et al.* 2016). Over the last decades, conservation efforts have focused on preserving the remaining populations, reducing extinction risks, and promoting recovery (Dulvy *et al.* 2016). Globally, there are five extant sawfish species (Faria *et al.* 2013, Last *et al.* 2016, Fricke *et al.* 2022): the narrow sawfish, *Anoxypristis cuspidata* (Latham, 1794); the largetooth sawfish, *Pristis pristis* (Linnaeus, 1758); the smalltooth sawfish, *Pristis pectinata* Latham, 1794; the dwarf sawfish, *Pristis clavata* Garman, 1906; and the green sawfish, *Pristis zijsron* Bleeker, 1851. All these species have experienced drastic declines in many coastal areas due to overfishing and habitat loss (Yan *et al.* 2021), becoming locally extinct in numerous regions in the last century (Dulvy *et al.* 2016, Ferretti *et al.* 2016). The International Union for Conservation of Nature (IUCN) classifies all five species as Critically Endangered (Carlson *et al.* 2022, Espinoza *et al.* 2022, Grant *et al.* 2022, Harry *et al.* 2022, Haque *et al.* 2023).

Reconstructing the historical zoogeography of sawfishes is challenging because of their long history of depletion, which often predates scientific monitoring. In the Mediterranean Sea, this situation is extreme, due to the much longer history of human impacts than in other global regions (Ferretti *et al.* 2016). Notwithstanding, the analyses of 21 museum specimens and 82 bibliographical records suggested that *P. pristis* and *P. pectinata* occurred in the Mediterranean Sea and went extinct before the 1970s (Ferretti *et al.* 2016). These findings challenged previous assumptions about sawfish biogeography, as the Mediterranean has been considered unsuitable for stable populations due to its limited estuarine systems, reduced shallow seagrass habitats, and seasonal temperature fluctuations compared to tropical nursery grounds (Ferretti *et al.* 2016). Yet, our understanding of sawfish habitat requirements remains poorly understood and are mainly inferred from two extant populations: *P. pectinata* in Florida (Poulakis *et al.* 2011, 2013, Simpfendorfer *et al.* 2011) and of *P. pristis* from northern Australia (Whitty *et al.* 2017). Most available information refers to nursery habitats in shallow coastal and estuarine environments, whereas the use of other essential habitats, such as feeding, breeding, or migratory areas, remains poorly documented (Simpfendorfer *et al.* 2011, Poulakis *et al.* 2013, Wueringer *et al.* 2023). These extant populations can still be studied experimentally but may not be representative of the ecological adaptations of extinct populations.

Due to sawfish rarity and poor conservation status, museum specimens such as dried rostra are crucial for obtaining biomolecular data to reconstruct their historical zoogeography and ecology (Phillips *et al.* 2009, Seitz and Hoover 2017). Over the past decade, several studies have highlighted the potential of rostra preserved in museums and private collections for species' identification, taxonomic revision, and provenance assessment (Fearing *et al.* 2018, Wiley *et al.* 2023, Wueringer *et al.* 2023, Smith *et al.* 2024, Fearing *et al.* 2025). However, historical records frequently lack complete information about the geographical origin of the individuals, the period when they were caught (e.g. collection date), and details about their acquisition process, which can be linked to the extensive trade of rostra that occurred historically among public and private collectors (Dulvy *et al.* 2016).

Morphometric analyses of rostra can efficiently identify sawfish specimens (Faria *et al.* 2013, Whitty *et al.* 2014, Seitz and Hoover 2017, Trif and Vonica 2018), especially if integrated with diagnostic molecular taxonomy methods (Moftah *et al.* 2011, Cariani *et al.* 2017, Petean *et al.* 2020, Bellodi *et al.* 2022, Carugati *et al.* 2022), which have already proven effective in discriminating among sawfish species (Phillips *et al.* 2009, Faria *et al.* 2013). Ancient DNA (aDNA) approaches are appropriate for analysing museum specimens and investigating historical evolutionary and ecological changes of rare or endangered populations (Orlando *et al.* 2021, Cilli *et al.* 2023). Specifically, these approaches can process tissues that were not originally collected and stored for molecular analyses, such as the cartilage and rostral teeth of sawfish specimens. Although these samples can yield less and more damaged DNA (degraded, fragmented, and contaminated by exogenous DNA; Pääbo *et al.* 2004, Puncher *et al.* 2019) than properly preserved ones (Wandeler *et al.* 2007), aDNA approaches have been successful in reconstructing the population history of several Mediterranean marine vertebrates, such as the Atlantic bluefin tuna, *Thunnus thynnus* (Puncher *et al.* 2019, Andrews *et al.* 2021) and the great white shark, *Carcharodon carcharias* (Leone *et al.* 2020).

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In this study, we present a replicable framework for reassessing sawfish rostra held in European museums and private collections. Our goals are: (i) to identify specimens to species-level using both molecular and morphometric tools; (ii) to assess the reliability of collection metadata and to identify potential Mediterranean records; and (iii) to evaluate the broader value of museum rostra for reconstructing the historical distribution of sawfishes. By doing so, we aim to provide a practical, integrative approach that museums and researchers can adopt to enhance the scientific utility of their collections.

MATERIALS AND METHODS

Inventory and confidence ranking of sawfish rostra

The inventorying of sawfish specimens was carried out by reviewing the existing literature to identify museums and private collections housing relevant material of Mediterranean origin (Ferretti *et al.* 2016). We then contacted curators and private owners to obtain additional information and access to sawfish specimens. In total, 33 museums and four private collections were contacted and provided access to sawfish rostra and associated data, including 2D scaled photographs, morphometric measurements, and rostral tissue samples (Supporting Information, Tables S1, S2). Efforts were also made to gather contextual information on specimens, including collection location and date (Supporting Information, Table S3). This information was primarily recovered from museum labels, catalogues, and archival documents, while for private collections (e.g. family heirlooms), anecdotal details were obtained from the owners. When precise collection years were unavailable, approximate date ranges were accepted.

To evaluate the reliability of collection metadata, we applied the method from Fearing *et al.* (2025). We assigned a confidence ranking (high, medium, low, or negligible) to each specimen by following a stepwise decision-making process (see fig. 1 and fig. S1 in Fearing *et al.* 2025 for a complete explanation and schematic assessment). The ranking was determined by evaluating key aspects of provenance documentation, including whether the data are connected to a primary source or not. Specifically, high confidence was assigned to specimens supported by original field tags or logbook entries documenting the collection event, i.e. direct primary sources. When information was available only through secondary sources, such as museum tags or catalogue records, the confidence level was reduced to medium, acknowledging the potential for transcription errors or incomplete documentation. If a specimen lacked a verifiable link to a primary source, making its provenance uncertain, it was classified as low confidence. Finally, when metadata included explicit uncertainty or when provenance information was entirely absent, the specimen was categorized as of negligible confidence, reflecting the highest level of uncertainty. To ensure objectivity, two independent assessors applied the ranking system to each specimen. In cases of disagreement, the final classification was assigned through consensus after discussion.

Molecular taxonomy

Tissue sampling

To obtain DNA from historical rostra without compromising their exhibition value, we selected the ventral surface at the base of the rostrum as the most suitable site for sampling cartilaginous tissue. This area is generally less visible in museum displays and allowed for minimally invasive sampling. Our choice was further supported by Phillips *et al.* (2009), who systematically tested multiple anatomical regions of dried sawfish rostra and identified the superficial skin as the most reliable source of amplifiable DNA. Before sampling the cartilage, rostra were superficially decontaminated with 1.5% sodium hypochlorite (bleach), and surface layers were removed with a slight abrasion with sandpaper. Small holes were performed with a drill to extract cartilage powder. All instruments were cleaned after each sampling event with bleach. We collected each sample into a sterile screw tube and stored it dry at room temperature until further laboratory analyses.

Laboratory procedures

All laboratory procedures followed high-sterility standards and appropriate criteria to prevent contamination by exogenous DNA (Cooper and Poinar 2000, Llamas *et al.* 2017, Cilli 2023). DNA extraction and pre-PCR (polymerase chain reaction) setup of 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), exclusively dedicated to ancient DNA analyses (Fulton 2012). 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). Reagents used during the DNA extraction and pre-PCR setup, and all the plastic labware, were exposed to UV radiation for 30 min 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) was worn during the analyses of ancient samples in the pre-PCR facility. Contamination was also ruled out by negative DNA extractions and PCR amplifications and conducting PCRs in a dedicated facility physically separated from pre-PCR procedures.

Total genomic DNA (gDNA) was extracted from 100 to 150 mg of tissue powder using the MinElute PCR Purification Kit (Qiagen, Hilden, Germany), following the chemical extraction protocol (Cilli *et al.* 2020) and accurately improved starting from previously published studies (Dabney *et al.* 2013). To ensure high-quality DNA recovery, the extracted gDNA was eluted in 50 µL of TET buffer (10 mMTris-HCl, 1 mM EDTA, 0.05% Tween-20). To verify the authenticity of the results, 25% of the samples were extracted and amplified following the same procedure, as previously conducted for this methodological approach (Ciucani *et al.* 2019).

Due to the highly fragmented nature of DNA from historical specimens, two overlapping amplicons (about 180–220 bp each) were identified from the nicotinamide dehydrogenase subunit 2 (*NADH2*) and two (about 150–200 bp) from the cytochrome *c* oxidase subunit I (*COI*) mitochondrial genes. These short regions have been designed to be consecutive in each mitochondrial marker to allow their combination into longer fragments. These regions were targeted by building two reference datasets: we retrieved all available to date *NADH2* and *COI* sequences belonging to the five sawfish species from both the BOLD systems (https://www.boldsystems.org) and the NCBI (http://www.ncbi.nlm.nih.gov/) online repositories (Supporting Information,

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Table S4). In addition, 92 mitogenomes of *P. pristis* by Feutry *et al.* (2015) were added to the datasets of the corresponding marker. Primer design and optimization of PCR conditions are given in Supporting Information, Text S1 and Table S5.

Sequence analysis

Forward and reverse trace files were checked and edited with MEGA v.11 (Tamura *et al.* 2021). The newly obtained sequences were added to the reference *NADH2* and *COI* datasets and were aligned using the ClustalW algorithm (Thompson *et al.* 1994) implemented in MEGA. 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 analyses. The NJ tree clustering (Saitou and Nei 1987) was obtained using MEGA with bootstrap support of 1000 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 a Markov chain Monte Carlo (MCMC) analysis conducted for two parallel runs with random starting trees, for 500000 generations 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 sequences of *Glaucostegus typus* (Glaucostegidae; GenBank code JN184059 and HQ955939 for *NADH2* and *COI*, respectively) were added to the final datasets as outgroups. Tree editing with bootstrap and posterior probability values was done in TreeGraph v.2 (Stöver and Müller 2010).

Morphometry of rostra

We employed two complementary morphometric approaches, linear morphometrics and landmark-based geometric morphometrics, to maximize the resolution and interpretability of rostral shape variation among sawfish species. While linear measures are powerful for species' classification and allow machine-learning techniques to perform well, they inherently reduce shape to univariate ratios and may miss spatial relationships among anatomical landmarks. On the other hand, geometric morphometrics captures the configurational geometry of rostra in a size- and orientation-independent manner, enabling visualization of subtle shape changes and increasing statistical sensitivity (Rohlf and Marcus 1993, Adams *et al.* 2004, Webster and Sheets 2010, Zelditch *et al.* 2012).

Linear measurements

Linear measurements (Fig. 1) were taken directly by the authors on each rostrum using a calliper, following protocols established in Faria *et al.* (2013), Whitty *et al.* (2014), Seitz and Hoover (2017), and Trif and Vonica (2018). The recorded measurements included: (i) total specimen length (TSL), (ii) total rostrum length (TRL), (iii) standard rostrum length (SRL), (iv) proximal rostrum width [PRW, equivalent to SRW in Whitty *et al.* (2014)], (v) distal rostrum width (DRW), (vi) proximal tooth gap (PTG), and (vii) distal tooth gap (DTG). For specimens lacking the rostrum–head junction, TRL was assumed equivalent to the maximum measurable rostrum length, as per Seitz and Hoover (2017). All variables were standardized as ratios relative to TRL to allow for size-independent comparisons, retaining SRL as an independent metric. Although SRL endpoints are generally less subjective than TRL (Whitty *et al.* 2014), we retained TRL in order to ensure comparability with previous datasets.

To assess the discriminatory power of rostral measurements among sawfish species, we applied a supervised machine-learning approach based on random forest (RF) classification (Breiman 2001). The training dataset included both rostra with molecular species' identification and reference specimens retrieved from previous studies ($N = 77$) for which homologous morphometric data were available. These references included museum and literature specimens with confirmed species' identity, representing *A. cuspidata* ($N = 12$), *P. pectinata* ($N = 25$), *P. pristis* ($N = 27$), and *P. zijsron* ($N = 11$), from published sources, including

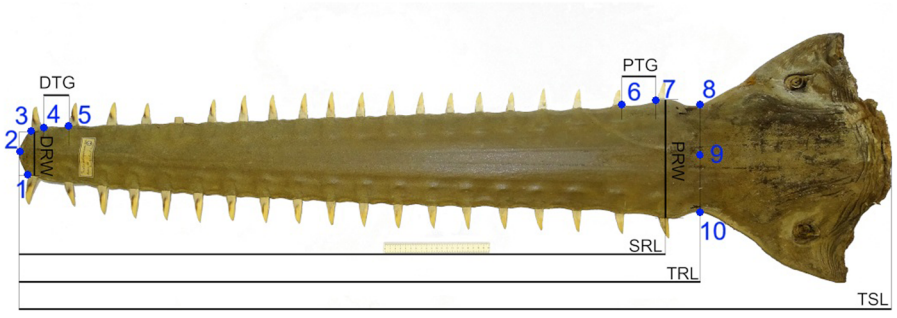

Figure 1. Linear measurements (in black) and Landmarks (in red) selected for the morphometric analyses of sawfish rostra. TSL, total specimen length; TRL, total rostrum length; SRL, standard rostrum length; PRW, proximal rostrum width; DRW, distal rostrum width; PTG, proximal rostral tooth gap; DTG,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

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Robillard and Séret (2006), Faria *et al.* (2013), Seitz and Hoover (2017), and Trif and Vonica (2018).

The RF model was implemented in R (R Core Team 2025) using the caret package (Kuhn 2008), applying a 10-fold cross-validation framework. Model optimization was performed by tuning the *mtry* parameter (i.e. number of variables randomly sampled at each node split) based on classification accuracy. Variable importance was extracted from the final model using the *varImp*() function. Once trained, the model was used to predict species' identity for rostra lacking molecular identification. Additionally, a principal component analysis (PCA) was performed on the standardized morphometric dataset to visualize patterns of variation and to assess clustering across species. The graph was visualized using the ggplot2 package in R (Wickham 2016).

After assignment to species-level, we performed two complementary analyses on rostrum measurements. First, descriptive statistics (range, mean, and standard error) of the five standardized linear measurements were calculated for each target taxon to enable comparison with other sawfish populations. Second, we estimated the total length (TL) of each individual to assess its maturity stage and to describe the overall size composition of the studied material. We used published relationships between rostrum dimensions and body length. For Australian sawfish species (*A. cuspidata*, *P. pristis*, and *P. zijsron*), TL was estimated from the SRL–TL relationships described by Whitty *et al.* (2014), following the approach of Wueringer *et al.* (2023). For the non-Australian sawfish *P. pectinata*, total length was estimated using the relationship between total rostrum length (TRL) and TL provided by Faria (2007). Individuals were considered juveniles if their estimated total length was below the species-specific maturity threshold: less than 2250 mm for *A. cuspidata*, and less than 3000 mm for *Pristis* species (Faria 2007, Thorburn *et al.* 2007, Peverell 2008, Morgan *et al.* 2011, Whitty *et al.* 2014).

Geometric morphometry

All available images of rostra were evaluated for their suitability for landmark-based morphometric analysis. Since photographs were obtained from different sources and devices, including those taken directly by the authors or provided by museum curators or private owners, focal length metadata were not available. In most photographs, a metric scale was placed adjacent to the rostrum and in the same plane, allowing for precise calibration. When a scale was not available, we used the measured distance between the two most distal rostral teeth for standardization. All images were included only if the rostrum was positioned flat and orthogonal to the camera sensor. In addition, photographs were visually inspected to exclude any with perspective distortion or lens' artefacts. This protocol ensured the comparability and reliability of morphometric data across all specimens, despite the heterogeneity in image sources and photographic equipment, in line with similar application of geometric morphometrics from archival or non-standardized images (Guillaud *et al.* 2016, Ibáñez and Jawad 2018, Bellodi *et al.* 2022).

Ten landmarks (Fig. 1) were selected a priori, focusing on the most variable region of the rostrum among sawfish species (Faria *et al.* 2013, Whitty *et al.* 2014, Seitz and Hoover 2017). These landmarks represent homologous points that can be reliably identified across all specimens. Landmarks were digitized using tpsDig2 v2.31 (Rohlf 2021), and a TPS (thin plate spline) file was created with tpsUtil (Rohlf 2015). The data were processed through a Procrustes' analysis using MorphoJ v1.07 (Klingenberg 2011). The RF model was implemented in R on Procrustes-aligned landmark coordinates, following the same 10-fold cross-validation workflow used for the linear morphometric dataset. The model was trained only on specimens with molecular IDs and subsequently used to predict species for the 'undetermined' rostra, allowing direct comparison with the linear morphometric results. Landmarks were then used to create a covariance matrix for a PCA and visualized with ggplot2 in R.

Integrating species' identification across methods

To assign each specimen to species-level, we integrated the results of molecular and morphometric analyses. When available, species' identification based on mtDNA (*NADH2* or *COI*) was considered the most reliable and was used as the final taxonomic assignment. For specimens lacking molecular data, species were assigned using two parallel RF models trained on linear measurements and on Procrustes-aligned landmarks, both calibrated with specimens of known identity. To validate morphometric classifications, we generated cross-validation confusion tables from both training datasets in R (caret package) to compare predicted and reference species' labels. When the two morphometric predictions disagreed, the assignment from the model with the higher mean cross-validated accuracy was retained as the final species' identification. All final species' assignments, whether molecular or morphometric, were compiled in a unified dataset used for subsequent analyses of geographic provenance and historical distribution patterns.

RESULTS

A total of 229 sawfish rostra were retrieved from museum and private collections (Supporting Information, Tables S1, S2), originally identified as *A. cuspidata* ($N = 34$), *P. pectinata* ($N = 44$), *P. pristis* ($N = 30$), *P. zijsron* ($N = 19$), *Pristis microdon* ($N = 5$), *Pristis marsilii* ($N = 1$), *Pristis antiquorum* ($N = 1$), and *Pristis* spp. ($N = 95$). However, *P. microdon* and *P. antiquorum* are synonyms of *P. pristis* (Faria *et al.* 2013), while *P. marsilii* probably resulted from an error in label interpretation or transcription.

Collection metadata (Supporting Information, Table S3) were often incomplete, with 168 specimens (73%) lacking geographic origin and 181 (80%) missing a recorded capture year. When available, geographic origin indicated the Mediterranean Sea ($N = 28$; Table 1), Indian Ocean ($N = 25$), Atlantic Ocean ($N = 5$), and West Pacific Ocean ($N = 3$). Capture years ranged from 1600 to 1980.

The standardized confidence ranking method was applied to all 229 specimens. Of these, specimens lacking collection location and dates were assigned a negligible confidence level and excluded from further distribution and temporal analyses. Among the remaining 61 specimens with available collection location, one was ranked as high confidence, 51 as medium, seven as low, and two as negligible (Fig. 2; Supporting Information, Tables S3, S6). Instead, of the 48 with information on the collection date, one was ranked as high confidence, 37 as medium, and 10 as low (Supporting Information, Tables S3, S6).

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Table 1. List of the 28 sawfish rostra labelled as Mediterranean, including available details on their geographic origin, year of capture, and integrated taxonomic identification as determined in this study. Whether specimens were previously inventoried by Ferretti *et al.* (2016) or included in other studies (reference column) is also indicated. The assigned confidence ranking (Fearing *et al.* 2025) is reported for each specimen (see also Fig. 2 and Supporting Information, Table S6 for the full dataset of evaluated specimens and the percent agreement).

Museum/collection	Museum label	Internal code ID	Location/site	Date/ time period	Integrated taxonomic identification	Reference	Location confidence ranking	Date confidence ranking
Civic Museum of Natural History, Milano, Italy	MNSM Pi 5102	MI203	Messina (Italy)	1962	*P. pectinata*		Medium	Medium
Comparative Anatomy collection, University of Bologna, Bologna, Italy	100944	B22	Adriatic		*P. pectinata*		Medium	Negligible
Museum of Natural History 'La Specola', University of Florence, Florence, Italy	6112	F7	Messina (Italy)	1837	*P. pectinata*	Vanni (1992); Ferretti *et al.* (2016)	Medium	Medium
Museum of Zoology, University of Navarra, Navarra, Spain	107 129	N78	Mediterranean Sea	Around 1900	*A. cuspidata*	Ferretti *et al.* (2016)	Medium	Medium
	107 127	N77	Mediterranean Sea	Around 1900	*P. zijsron*	Ferretti *et al.* (2016)	Medium	Medium
	107 145	N79	Mediterranean Sea	Around 1900	*P. zijsron*	Ferretti *et al.* (2016)	Medium	Medium
Museum of Zoology, University of Padoa, Padoa, Italy	P29e	P58	Adriatic	<1730	*P. pectinata*	–	Medium	Low
	P28e	P59	Adriatic	<1730	*P. zijsron*	–	Medium	Low
Natural History Museum, Dubrovnik, Croatia	PMD 20	C74	Adriatic		*P. zijsron*	Ferretti *et al.* (2016)	Medium	Negligible
Civic Museum Foundation, Rovereto (Trento), Italy	PES 00018	R66	Venice	1900	*A. cuspidata*	Ferretti *et al.* (2016)	Medium	Medium
	PES 00019	R67	Genoa	1900	*A. cuspidata*	Ferretti *et al.* (2016)	Medium	Medium
	PES 00020	R64	Genoa	1900	*P. zijsron*	Ferretti *et al.* (2016)	Medium	Medium
Natural History Museum, Split, Croatia	PMST 10	C71	Southern Adriatic	1901	*P. zijsron*	Ferretti *et al.* (2016)	Medium	Medium
Natural History Museum, University of Pisa, Calci (Pisa), Italy	Pe 0116	Pi60	Mediterranean Sea		*A. cuspidata*	Ferretti *et al.* (2016)	Medium	Negligible
Wilderness s.n.c Studi Ambientali, Palermo, Italy		BZ104	Mediterranean Sea		*P. pristis*		Negligible	Negligible
Zoological Collection 'Giuseppe Scarpa', Treviso, Italy	MSGZ983/MP	TR180	Mediterranean Sea		*A. cuspidata*		Low	Negligible
	MSGZ981/MP	TR181	Mediterranean Sea		*A. cuspidata*		Low	Negligible
	MSGZ4846/MP	TR179	Mediterranean Sea		*P. pristis*		Low	Negligible
	MSGZ980/MP	TR183	Mediterranean Sea		*P. pristis*		Low	Negligible
	MSGZ3442/MP	TR177	Mediterranean Sea		*P. zijsron*		Low	Negligible
	MSGZ3459/MP	TR178	Mediterranean Sea		*P. zijsron*		Low	Negligible
	MSGZ982/MP	TR182	Mediterranean Sea		*P. zijsron*		Low	Negligible
Zoological Museum 'P. Doderlein', University of Palermo, Palermo, Italy	AN182	Pal109	Palermo (Italy)		*A. cuspidata*		Medium	Negligible
	AN184	Pal110	Palermo (Italy)		*P. pectinata*	Doderlein (1881)	Negligible	Negligible

(Continued)

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Table 1. Continued.

Museum/collection	Museum label	Internal code ID	Location/site	Date/ time period	Integrated taxonomic identification	Reference	Location confidence ranking	Date confidence ranking
Museum of Natural Sciences, Barcelona, Spain	MZB920265	BCN226	Mediterranean Sea		*P. pristis*		Medium	Negligible
	MZB825331	BCN230	Mediterranean Sea		*P. pristis*	Ferretti *et al.* (2016)	Medium	Negligible
	MZB825327	BCN228	Mediterranean Sea		*A. cuspidata*	Ferretti *et al.* (2016)	Medium	Negligible
	MZB825329	BCN229	Mediterranean Sea		*P. pristis*	Ferretti *et al.* (2016)	Medium	Negligible

Molecular identification of rostral specimens

We could obtain suitable tissue for biomolecular analyses from 130 rostra out of the 229 collected. Species' assignment was achieved for all 130 rostral specimens, based on either *NADH2* (six specimens), *COI* (four), or both markers (120). Overall, we obtained 126 individual sequences of *NADH2* and 124 of *COI* gene fragments, excluding four *NADH2* and six *COI* sequences, respectively, due to the low quality of trace files. Final consensus sequences were 350 bp and 320 bp in total length for *NADH2* and *COI* datasets, respectively.

According to the concordant molecular taxonomy results, we identified 33 rostra as *A. cuspidata*, 17 as *P. pectinata*, 19 as *P. pristis*, and 61 as *P. zijsron*. The *NADH2* and *COI* phylogenetic trees showed well-defined species-specific clusters with high support of bootstrap and posterior probabilities in which sequences from historical specimens were bundled with contemporary references (Fig. 3). Species-specific bootstrap values were equal to 99% and 100% in the *NADH2* NJ tree, while they ranged from 96% (*P. zijsron*) to 100% in the *COI* NJ tree. Posterior probabilities ranged from 0.9 (*P. pectinata* and *P. zijsron*) to 1.0 in the *NADH2* BI tree and were equal to 1.0 in all clusters in the *COI* reconstruction. Relatively low bootstrap values were obtained in the ML trees, ranging from 71% (*P. pectinata*) to 100% for *NADH2* and from 48% (*P. zijsron*) to 100% for *COI*.

Morphometry of rostra

We obtained linear measurements from 226 rostra out of the 229 collected (Table 2; Supporting Information, Table S7; three rostra were incomplete, but included in the molecular analysis). The RF model trained on 204 rostra with known species' identity (127 genetically identified + 77 reference public specimens) showed high classification performance, achieving an average cross-validated accuracy of 95.5% (±0.060 SD) and a Cohen's Kappa of 0.94 (±0.081 SD; *mtry* = 2). Among all predictor variables, the distal tooth gap (DTG) and the proximal rostrum width (PRW) contributed most to species' discrimination (79.94% and 71.76% respectively; Supporting Information, Fig. S1). This optimized model was then applied to the 99 rostra with no prior species' assignment. The PCA on all 303 rostra (77 known references and 226 from this study: 127 molecular identified and 99 predicted) revealed that the first two principal components accounted for 86.1% of total variance, effectively separating the four target species. While clusters were generally well defined, a partial overlap was observed between *P. zijsron* and *P. pectinata* (Fig. 4A), consistent with the RF training predictions. According to the variable-importance analyses on the training dataset, the PCA loadings indicated that PC1 was mainly driven by DTG, PRW, and distal rostrum width (DRW), while PC2 reflected variation in the proximal tooth gap (PTG).

A total of 208 rostra pictures were suitable for the digitalization of landmarks and were retained for geometric morphometric analysis. The RF model trained on the Procrustes-aligned landmark coordinates achieved an average 10-fold cross-validated accuracy of 91.8% (SD = 0.067) and a Cohen's Kappa of 0.87 (SD = 0.106; *mtry* = 2). According to the variable importance scores, landmark 7, located in the proximal portion of the rostrum, showed the highest contribution to species' discrimination (75.13%; Supporting Information, Fig. S1), followed by landmarks 10 and 6. The PCA computed on all 208 specimens (Fig. 4B), revealed patterns largely consistent with those observed in the linear morphometric PCA (Fig. 4A), with a clear

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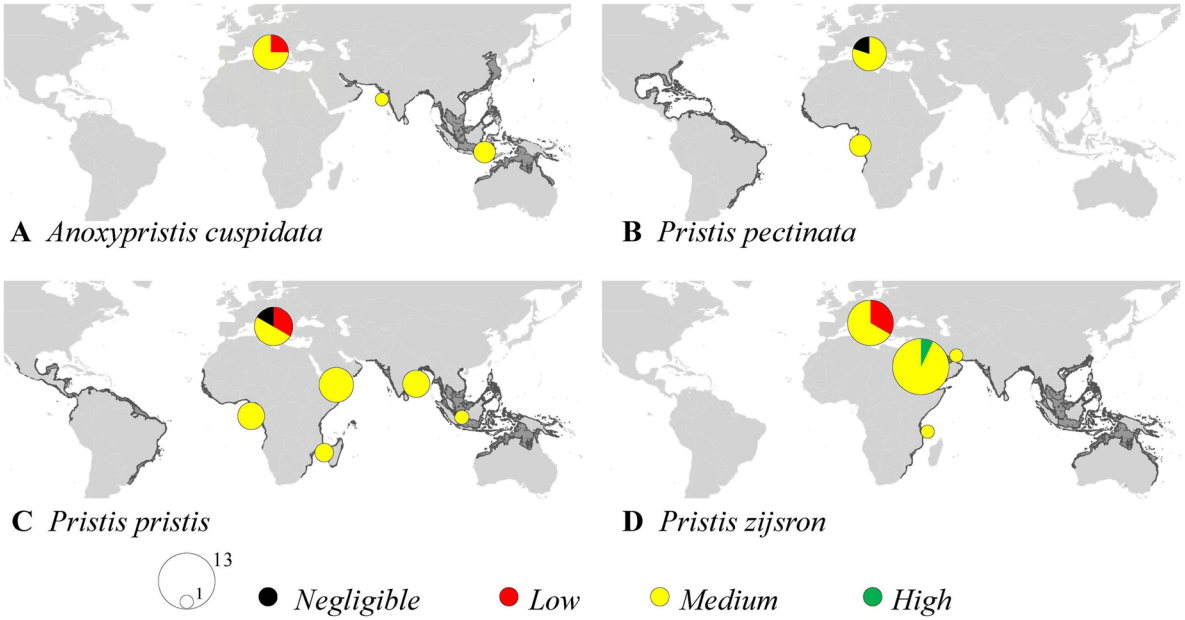

Figure 2. Map showing the historical range of 61 sawfish rostra with collection location data available, compared to the IUCN Red List assessment range (dark grey). Circle size represents the number of specimens from each location, while colours indicate the proportion of confidence rankings, illustrating the reliability of provenance data.

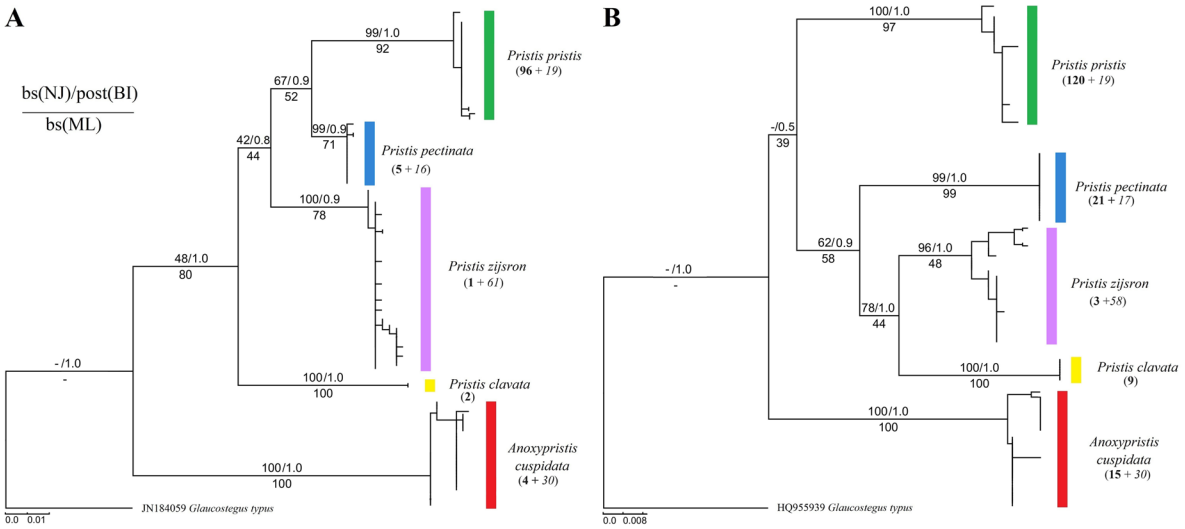

Figure 3. Phylogenetic trees of sawfish species reconstructed from mitochondrial *NADH2* (A) and *COI* (B) gene fragments using neighbour-joining (NJ), Bayesian inference (BI), and maximum likelihood (ML) methods. Node support is indicated by NJ and ML bootstrap values (bs) and Bayesian posterior probabilities (post). Vertical bars next to terminal branches are colour-coded by species, consistent with Figure 4. Numbers in parentheses indicate the number of sequences obtained from public databases (**bold**) plus sequences retrieved from rostrum specimens (*italic*) for each species. See Supporting Information, Table S4 for a complete list of reference sequences retrieved from public databases.

separation among the four species along the first principal component (86.33% of total shape variation), and a marked overlap between *P. zijsron* and *P. pectinata*. Inspection of loadings indicated that PC1 was mainly driven by landmarks 6 and 7 located at the base of the most proximal rostral teeth, while PC2 reflected shape variation where the rostrum begins to flare (landmark 10).

Integrated species' identification

The identification of historical sawfish rostra using a combination of DNA sequencing with morphometrics classified 104 specimens as *P. zijsron*, 52 as *A. cuspidata*, 47 as *P. pristis*, and 26 as *P. pectinata*. Of these, 95 rostra had not been previously identified at the species level, while 43 were misidentified. Among the unidentified specimens, we assigned 50 individuals to *P. zijsron*, 17 to *P. pristis*, 13 to *P. pectinata*, and 15 to *A. cuspidata*. Notably, among the 43 misidentified specimens, 32 cases involved misclassifications between *P. pectinata* and *P. zijsron*.

A total of 130 specimens had molecular identification, which was prioritized whenever available. These identifications were used to train both the linear morphometric and geometric morphometric

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Table 2. Sample size (*N*) and descriptive statistics of the five standardised measures on the 226 sawfish rostra (given as percentages of TRL; SRL, standard rostrum length; PRW, proximal rostrum width; DRW, distal rostrum width; PTG, proximal rostral tooth gap; DTG, distal rostral tooth gap) for each sawfish species. The estimated total length (TL) of animals is expressed in mm and follows the relation between rostrum length and total animal length by Faria (2007) and Whitty *et al.* (2014).

Species	*N*		SRL	PRW	DRW	PSD	DSD	Estimated TL
Anoxypristis cuspidata	51	Mean ± SD	78.49 ± 4.33	8.42 ± 0.72	5.02 ± 0.75	4.12 ± 0.63	1.08 ± 0.26	2287.06 ± 588.85
		Min	68.75	6.94	4.00	2.88	0.69	910.00
		Max	88.32	10.00	7.26	5.43	1.67	3450.00
Pristis pectinata	24	Mean ± SD	92.06 ± 4.24	14.34 ± 1.27	7.39 ± 1.23	5.35 ± 0.69	2.05 ± 0.41	2205.57 ± 1190.39
		Min	78.74	12.33	5.49	3.35	1.54	712.50
		Max	97.62	17.35	10.96	6.47	3.20	3799.96
Pristis pristis	47	Mean ± SD	93.71 ± 2.87	19.04 ± 1.68	7.76 ± 0.91	5.46 ± 0.54	3.69 ± 0.51	3774.75 ± 1192.68
		Min	85.45	14.84	6.07	4.24	2.46	730.43
		Max	99.12	21.94	10.45	7.27	4.62	5500.00
Pristis zijsron	104	Mean ± SD	91.12 ± 3.41	10.87 ± 1.23	5.29 ± 1.05	6.14 ± 0.93	1.18 ± 0.30	3900.16 ± 1280.62
		Min	84.75	8.13	3.65	4.02	0.61	637.50
		Max	98.21	14.37	9.09	8.52	2.18	5604.17

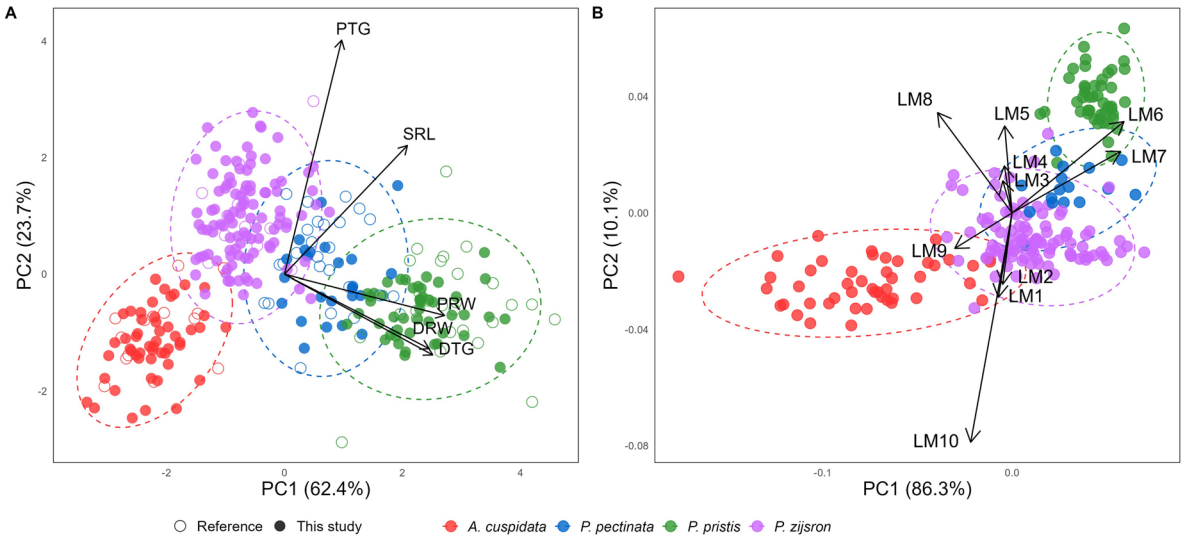

Figure 4. Principal component analyses (PCA) of sawfish rostra based on two complementary morphometric approaches. (A) PCA based on standardized linear measurements of 303 rostra (127 genetically identified, 99 morphometrically assigned, and 77 reference specimens). (B) PCA based on 10 anatomical landmarks digitized from 208 rostra images analysed via geometric morphometrics. Species are colour-coded as in Figure 3. Ellipses represent 95% confidence intervals around group centroids.

RF models. Within the linear morphometric training dataset, nine specimens were misclassified (Table 3A), including three from this study that showed discrepancies between molecular and predicted species' identity: two juvenile *P. zijsron* specimens (T114 and T116; Supporting Information, Table S2) predicted as *P. pectinata*, and one *P. pectinata* specimen (BZ97; Supporting Information, Table S2) predicted as *P. pristis*. In the geometric morphometric training dataset, 10 specimens showed discrepancies between molecular and predicted species' identity (Table 3B), mainly involving reciprocal misclassifications between *P. pectinata* and *P. zijsron* (BZ90, BZ98, RM88, T34, Vo133, T114, T116; Supporting Information, Table S2). Additional incongruences included two *A. cuspidata* specimens (T43 and T52) predicted as *P. zijsron* and one *P. pectinata* specimen (BZ101) predicted as *P. pristis*.

For the remaining 99 rostra lacking genetic data, species were assigned using two parallel RF models based on linear and geometric morphometrics. The two classifiers produced largely concordant results, with only seven specimens showing discrepancies between linear and geometric predictions (Supporting Information, Table S2), including three juveniles. Most inconsistencies involved reciprocal classifications between *P. zijsron* and *P. pectinata* (F15, GE205, PV184, Ve152), while additional mismatches included two specimens identified as *P. pectinata* by the geometric model and as *P. pristis* by the linear model (B139, BCN230), and one disagreement between *P. zijsron* (geometric) and *A. cuspidata* (linear) (N219). In all discordant cases, the final species' assignment followed the linear morphometric prediction, corresponding to the model with the higher overall accuracy.

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Table 3. Cross-validation confusion tables for the RF models trained on (A) standardized linear morphometric measurements (LM) and (B) Procrustes-aligned geometric morphometric landmarks (GM).

A	Reference				
LM Prediction	*A. cuspidata*	*P. pectinata*	*P. pristis*	*P. zijsron*	**Total**
A. cuspidata	45	0	0	1	
P. pectinata	0	37	1	3	
P. pristis	0	0	45	0	
P. zijsron	1	3	0	68	
Total (N)	46	40	46	72	204

B	Reference				
GM Prediction	*A. cuspidata*	*P. pectinata*	*P. pristis*	*P. zijsron*	**Total**
A. cuspidata	29	0	0	0	
P. pectinata	0	6	0	2	
P. pristis	0	1	18	0	
P. zijsron	2	5	0	57	
Total (N)	31	12	18	59	120

Rows indicate predicted species and columns the reference species (molecularly confirmed or validated references).

Mediterranean-labelled specimens

Among the 28 specimens labelled as originating from the Mediterranean (Table 3), 13 corresponded to museum specimens previously inventoried and verified by Ferretti *et al.* (2016) as part of their Mediterranean museum exhibit list [see table 1 in Ferretti *et al.* (2016)]. All were further assessed to determine the reliability of their collection metadata. None were ranked as high confidence, while the majority (19) received a medium confidence ranking. Seven specimens were classified as low confidence, typically due to the absence of museum tags, although provenance information was available through curatorial input. Two specimens received a negligible confidence ranking (PAL110 and BZ104) as they incorporated ambiguous wording, such as 'probably', within the catalogue description or the provenance reconstruction provided by the owner. This subset included nine specimens identified as *P. zijsron* (six medium, three low confidence); eight as *A. cuspidata* (six medium, two low); six as *P. pristis* (three medium, two low, one negligible); and five as *P. pectinata* (four medium, one negligible) (Fig. 2).

Regarding the date confidence ranking, only 11 specimens had any temporal information available: of these, two were assigned low confidence (based on curator reconstructions without original record dates), and nine were assigned medium confidence, as the only temporal reference derived from the museum label.

DISCUSSION

The conservation of sawfishes remains a global challenge (Dulvy *et al.* 2016, Yan *et al.* 2021), with most research and recovery efforts concentrated in a few regions, such as Australia (e.g. Morgan *et al.* 2015, 2017, 2021, Whitty *et al.* 2017, Kyne *et al.* 2021, Lear *et al.* 2023) and Florida (Graham *et al.* 2021, Smith *et al.* 2021). Other regions remain poorly investigated despite evidence of former sawfish presence (e.g. Leeney and Poncelet 2015, Reis-Filho *et al.* 2016, Bonfil *et al.* 2017, 2018, 2021, 2024, Jabado *et al.* 2017, White *et al.* 2017, Elhassan 2018, Haque and Das 2019, Haque *et al.* 2020, Cabanillas-Torpoco *et al.* 2023, Rubio-Cisneros *et al.* 2023).

Natural history museum collections provide a valuable resource to address these gaps, allowing for retrospective reconstructions of species' diversity and distribution in data-poor regions. Given that many museum rostra lack precise collection metadata, we applied a workflow that integrates targeted tissue sampling and molecular sequencing (Phillips *et al.* 2009, Ciucani *et al.* 2019) with morphometric classification (Fearing *et al.* 2018, Wiley *et al.* 2023, Wueringer *et al.* 2023, Smith *et al.* 2024) and metadata confidence scoring (Fearing *et al.* 2025) to reassess and validate sawfish specimens across institutions. Our integrated approach provides a practical, minimally invasive, and replicable framework for the identification of all 229 rostra and contributes to refining species-level records of four sawfish species in these historical collections. This represents one of the most comprehensive assessments to date of historical collections of sawfish rostra combining molecular and morphometric data within the same analytical framework. By including specimens with genetically confirmed identities, this dataset enables a direct and quantitative evaluation of the reliability of morphometric identification. Both RF models, based on linear and geometric morphometrics, showed high classification performance (95.5% and 91.8%, respectively) and were, therefore, used to assign species' identity to the 99 rostra lacking molecular data. Among these, the two morphometric classifiers produced largely concordant results, with only seven cases of disagreement. Four of these involved reciprocal predictions between *P. zijsron* and *P. pectinata*, including three juvenile specimens. These few mismatches further highlight the need for caution when classifying juvenile rostra based solely on morphometric traits, as previously noted in other studies (Whitty *et al.* 2014, Wueringer *et al.* 2023). The partial overlap between *P. zijsron* and *P. pectinata* observed in the PCA reflects this pattern of morphological continuity and is more comparable to the results of Seitz and Hoover (2017) than to Whitty *et al.* (2014), who examined the four Australian *Pristis* species and, therefore, did not include *P. pectinata*. This approach is especially valuable when genetic material is degraded, absent, or inaccessible, as our results demonstrate that standardized morphometric analyses can achieve species-level accuracy comparable to DNA-based identifications. Such methodological robustness highlights the potential of morphometric classification as a cost-effective and non-destructive tool for revising museum-held sawfish collections, particularly in contexts where molecular analyses are unfeasible. Beyond sawfishes, this integrative framework could be adapted to other elasmobranchs or morphologically similar taxa, particularly when dealing with cryptic species, incomplete specimens, or uncertain historical records (Iacovelli *et al.* 2025).

Given the precarious conservation status of sawfishes, it is essential to evaluate all available historical evidence, even if incomplete. This principle underpins our approach and should guide future research on historical species' distributions, especially

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when formal monitoring data are lacking. Among the 28 specimens labelled as Mediterranean (Table 1), four species were identified: *P. pectinata* (five), *P. pristis* (six), *P. zijsron* (nine), and *A. cuspidata* (eight). While *P. pristis* and *P. pectinata* have historical records in the Mediterranean and adjacent Eastern Atlantic (Ferretti *et al.* 2016), the presence of *P. zijsron* and *A. cuspidata* among specimens labelled as Mediterranean requires careful interpretation. Although fossil evidence suggests that *A. cuspidata* may have occurred in the region during the Late Neogene (>2.58 Mya) and Early Pliocene (<5.33 Mya) (Pawellek *et al.* 2012, Collareta *et al.* 2017), no fossil or historical records currently supports the presence of *P. zijsron* in the Mediterranean. Its known range is limited to the West Pacific Ocean and the Red Sea (Dulvy *et al.* 2016). Although the historical presence of sawfishes in the Mediterranean remains debated, and current distribution maps do not include this region (Carlson *et al.* 2022, Espinoza *et al.* 2022, Harry *et al.* 2022, Haque *et al.* 2023), our findings demonstrate how museum-held rostra, when assessed through an integrated framework of species' identification and metadata validation, can help to distinguish credible records from those affected by uncertain provenance. In this context, the integration of robust species-level identification with standardized provenance assessment (Fearing *et al.* 2025) provides a transparent framework to discriminate credible records from those affected by ambiguous labelling or trade. Such validated baselines are crucial for reconstructing extinction timelines, informing global Red List reassessments, and improving the reliability of occurrence data used in conservation planning (Dulvy *et al.* 2016, Kyne *et al.* 2021). Confidence-ranking assessments revealed that many Mediterranean-labelled specimens had uncertain provenance, as their reported geographic origin derived from secondary sources such as museum labels or catalogues. Medium-confidence cases corresponded to specimens with plausible but undocumented Mediterranean origin (e.g. lacking field notes or collection tags). Similarly, the few specimens with reported collection dates were mostly ranked as medium confidence. These records represent the most reliable information currently available, highlighting the uncertainty inherent in historical archives. Despite these limitations, rostra preserved in collections remain invaluable for reconstructing past biodiversity, provided that metadata gaps and inconsistencies are explicitly documented (Whitty *et al.* 2014, Fearing *et al.* 2025). Variability in curatorial practices over time, including reliance on secondary or ambiguous information (Verry *et al.* 2019), further complicates provenance reconstruction. When reliable, however, chronological data can also offer insights into historical population and distribution patterns, as recently demonstrated for *P. pectinata* by Smith *et al.* (2024).

Another factor contributing to mislabelling and taxonomic inconsistencies is the historical evolution of sawfish classification. *Pristis zijsron* was formally described only in 1851, and in the early 20th century, access to updated taxonomic literature was limited, leading to persistent misidentifications in museum records. For example, several 19th-century studies of Red Sea fish fauna referred to *P. zijsron* as *Pristis pectinatus* (Rüppell 1837, Klunzinger 1871). Similarly, in the Brukenthal National Museum of Sibiu (Romania), eight rostra collected from Singapore and Ethiopia in the late 19th century were originally catalogued as *Pristis pectinatus*, but six were later identified as *P. zijsron* and two as *A. cuspidata* (Trif and Vonica 2018). This issue is further reflected in our study, where 43 rostra were found to be misidentified, with a predominant trend of misclassification between *P. pectinata* and *P. zijsron* (32 cases). These findings highlight how taxonomic inconsistencies may obscure historical records and complicate efforts to reconstruct species distributions.

Although some labels may appear to support a possible historical occurrence of sawfish in the region, the absence of independent capture records and high-confidence provenance suggests these are more plausibly explained by mislabelling or uncertain origin. Beyond their uncertain provenance, however, these Mediterranean-labelled rostra expand the morphological and molecular reference dataset available for Pristidae, improving species' delimitation and supporting future identification of unclassified material. Given these limitations, a systematic revision of historical sawfish records is necessary. In some regions, a single capture or a museum specimen is taken as sufficient evidence for historical extinction (Hudgins *et al.* 2020), whereas in others, multiple records have been considered insufficient to confirm past presence (Yan *et al.* 2021). These inconsistencies highlight the complexity of reconstructing sawfish population histories, as many declines predated scientific monitoring. These challenges are not confined to the Mediterranean. Globally, historical trade of sawfish rostra, combined with inconsistent museum documentation, has obscured past distributions worldwide (Hoover 2008, Dulvy *et al.* 2014). The adoption of standardized provenance assessment methods, such as the decision-making approach proposed by Fearing *et al.* (2025) will be essential to distinguish genuine historical occurrences from artefacts of trade or curatorial bias. Complementary techniques, including radiocarbon and stable isotope analyses, could further refine provenance reconstructions. While not yet applied to sawfish rostra, similar isotopic studies on well-preserved skeletal material from other marine vertebrates have provided long-term evidence of trophic and ecological shifts (Christensen and Richardson 2008, Andrews *et al.* 2023) and insights into habitat preferences (Bevacqua *et al.* 2021, Pasino *et al.* 2026). These approaches, when preservation allows, could substantially enhance the reconstruction of historical sawfish biogeography.

CONCLUSION

In this study, we conducted a comprehensive re-evaluation of 229 sawfish rostra, integrating molecular and morphometric analyses to achieve robust species-level identifications and refine historical records for four sawfish species. This integrative framework demonstrates that morphometric approaches can achieve accuracy comparable to DNA-based identifications, providing a practical and replicable method for museum-held material where genetic data are unavailable or degraded.

In parallel, we independently assessed the reliability of associated collection metadata, including 28 rostra labelled as Mediterranean. None met high-confidence provenance criteria, while several medium-confidence cases suggest that sawfishes may once have occurred in the region, although current evidence remains inconclusive. This distinction between species' identification and provenance evaluation is crucial to avoid over-interpretation of archival material.

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Our workflow should be widely adopted, not only in regions with confirmed historical sawfish occurrences but also in peripheral areas where evidence remains scarce. Historical ecology approaches often yield unexpected insights, helping to reconstruct ecological baselines and species' historical ranges (McClenachan *et al.* 2012). Given that sawfishes rank among the most endangered marine species, critically reassessing all available historical evidence, even when incomplete, remains essential to advance understanding of their past distributions and to inform ongoing conservation efforts.

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SUPPLEMENTARY DATA

Supplementary data is available at *Zoological Journal of the Linnean Society* online.

CONFLICT OF INTEREST

We declare no conflicts of interest.

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DATA AVAILABILITY

The sequence data that support the findings of this study are openly available in Zenodo at 10.5281/zenodo.14016799. Linear measures are available in supporting information (Table S7) while pictures of rostra that support the findings of this study can be requested from the corresponding author.

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