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The sawfish (Rhinopristiformes, Pristidae) rostrum displayed in the "Basilica Santuario del Carmine Maggiore" in Naples, Italy: A long story of legends and taxonomic errors

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Abstract

Although historically widespread in most of the shallow and warm waters of the world, the presence of sawfishes in the Mediterranean Sea is still a debated question. For some authors, they never inhabited this basin except as vagrants, while for other authors both *Pristis pristis* and *Pristis pectinata* were present in the Mediterranean Sea but were extirpated in the 1960s–1970s. The sawfish rostrum kept in the Basilica Santuario del Carmine Maggiore (Naples, Italy), and considered by some authors the first record of sawfish in the Mediterranean was studied using a combination of morphological, genetic, radiocarbon dating and histochemical staining methods to validate the taxonomic identification, estimate its age and assess its geographical origin. Results indicate that the rostrum does not belong to *P. pristis* as reported by previous authors, but instead possesses morphological and genetic characters typical of *P. pectinata*. In addition, the radiocarbon age shows that the rostrum is more recent than previously believed, dating it back to the mid-nineteenth century, and genetic and meristic results cast doubt on its presumed Mediterranean origin. This study demonstrates that historical records should always be critically evaluated before using them to draw any far-reaching conclusion about species' past ecology and/or biogeography, and that future studies using historical information and specimens should adopt an integrative taxonomy approach similar to the one used here.

Keywords

batoids – endangered species – historical DNA – Mediterranean Sea – *Pristis* – taxonomy

Introduction

Although historically widespread in most of the shallow and warm seas of the world (Faria et al., 2013; Last et al., 2016), the presence of sawfishes (order Rhinopristiformes, family Pristidae) in the Mediterranean basin remains highly debated. Largetooth sawfish *Pristis pristis* (Linnaeus, 1758) and smalltooth sawfish *Pristis pectinata* Latham, 1794 were included (often as uncertain) in historical and recent regional faunal lists (Rafinesque, 1810; Giglioli, 1880; Carus, 1893; Tortonese, 1956; Stehmann & Bürkel, 1984; Fischer et al., 1987; Quignard & Tomasini, 2000; Serena, 2005; Bariche, 2012). Furthermore, Psomadakis et al. (2009) reported the finding of an old museum specimen and archive data possibly supporting the presence of a *Pristis* in the Gulf of Naples, Italy. In a broader paper dealing with the Mediterranean fish biodiversity, Psomadakis et al. (2012) make a remark on the above finding

but exclude *Pristis* spp. from the Mediterranean fish inventory. In a global synopsis of the rays of the world, Last et al. (2016) considered only *P. pectinata* as part of the Mediterranean fish fauna and stated that the species is presently extinct through most of its original distribution. However, in a recent review of sawfish species delineation and global population structure, Faria et al. (2013) suggested that sawfishes had never formed resident, breeding or core populations in the Mediterranean basin. This view was shared in the latest IUCN global sawfish conservation strategy workshop (London, May 2012), where it was concluded that the Mediterranean Sea was not part of the sawfish's historical distribution range (Harrison & Dulvy, 2014; see also Tortonese, 1982; Stehmann & Bürkel, 1984; Tortonese, 1985; Tortonese, 1987; Bilecenoglu et al., 2002; Dulvy et al., 2016a, for similar considerations). This conclusion was based on the perception that reliable evidence of sawfish historical occurrences in the basin is lacking, as well as information derived from three additional lines of reasoning: 1) a large portion of Mediterranean coastal waters are seasonally too cold to host stable sawfish populations; 2) no sawfish records are available from Mediterranean areas with the most suitable environmental conditions for sawfish (e.g., the Nile delta); 3) the Mediterranean has been a global-trade crossroad for millennia, and thus, museums or personal exhibits of sawfish from the region are possibly non-Mediterranean specimens acquired from curio markets.

On the contrary, management (EC, 2022) and conservation (Kyne, 2015; Dulvy et al., 2016b; Kyne, 2016a, b) organisations operating in the Mediterranean still enlist sawfishes as part of the regional fish fauna (Pristidae are also included in Appendices I and II of the Convention on the Conservation of Migratory Species of Wild Animals (CMS) Bern, 2015), and some authors (Ferretti et al., 2016; Serena et al., 2020), argue that sawfishes were stable inhabitants of the Mediterranean Sea and were definitively extirpated during the 1960s and 1970s. Their conclusions are based on historical bibliographic references and museum specimens which, by the admission of the authors, are not entirely reliable and in some cases are even defined as "anecdotal". In addition, the authors conclude that the Mediterranean Sea has been inhabited in the past by both sawfish species, *P. pristis* exclusively occurring in the western Mediterranean and *P. pectinata*, with a wider distribution range, present also in the Eastern basin with a discontinuous and patchy distribution. They also claim that the sawfish rostrum kept as a relic in the Basilica Santuario del Carmine Maggiore (Naples, Italy; hereinafter the "BSCM") represents the first sawfish record in the Mediterranean Sea (see also Ferretti, 2014). In a broader historical discussion on the past presence of cartilaginous fishes in the Mediterranean, Mojetta et al. (2018) addressed this issue by citing the above rostrum as a possible example of the first documented record of a sawfish in the Mediterranean.

Based on some old stories and legends reported by 16th century Neapolitan writers and historians (e.g., Costo, 1588), the above sawfish rostrum was connected by Costa (1853a) to a supposedly "miraculous event" that took place in August 1573. The story is set during the Kingdom of Spain's war against the Ottoman Empire for the control of the Western Mediterranean and tells about a Spanish galleon that left Naples, for a military expedition to Tunis that almost sank off Messina in the southern Tyrrhenian Sea due to a leak in the hull. According to the original text, an unidentified fish entered through the leak and plugged it, preventing water from entering and thus sinking the ship. In addition to connecting the sawfish rostrum held in the BSCM to the above story, Costa (1853a) provides a detailed morphological description and attributes the rostrum to an individual of *Pristis antiquorum* Latham, 1794, a species presently considered to be a synonym of *P. pristis*.

This study uses morphological and genetic analyses to validate species identification of the sawfish rostrum held at the BSCM. For this scope, histological analysis of rostral cartilage was carried out to evaluate its conservation status and suitability for genetic analysis. In addition, radiocarbon dating and the comparison of genetic results with those previously published (Faria et al., 2013) were used to confirm age and geographical origin extrapolated from the legend surrounding this important relic of the city of Naples.

Material and methods

Morphology and meristics

The BSCM sawfish rostrum (fig. 1A) was measured as shown in fig. 2A according to protocols used in previous studies (Robillard & Séret, 2006; Faria et al., 2013; Whitty et al., 2014; Seitz & Hoover, 2017) with some modifications due to its conservation status. Total length (TL) of the specimen bearing the rostrum kept in the BSCM was estimated by multiplying the total rostral length (TRL) by 3.91 (see Faria et al., 2013). All measurements were made to the nearest mm using a digital caliper or measuring tape, depending on the size of the individual characters. Rostral tooth count includes teeth and empty alveoli. Given that the sawfish rostrum under investigation is broken at the tip and the rostrum-head juncture is not visible, the total rostrum length (TRL) was measured from the mid-point of its tip to the mid-point of its basal-most edge. In addition, below the level of the second pair of teeth (only alveoli visible), the rostrum is cut on

FIGURE 1 (A) Picture of the sawfish rostrum kept in the BSCM and (B) illustration of the "miraculous event" in which it allegedly took part; note the fish tail protruding from the leak circled in white (from page 81; Bacco, 1605). (C) The original lacquer wax stamp present on the rostrum (central) compared to the logo of the Roman Curia of the Carmelite Order (left) and the logo of the Carmelite fathers of Naples (right). The logo of the Roman Curia of the Carmelite Order was downloaded from https://fraticarmelitani.wixsite.com/fraticarmelitani and the logo of the Carmelite fathers of Naples was photographed by N. Maio.

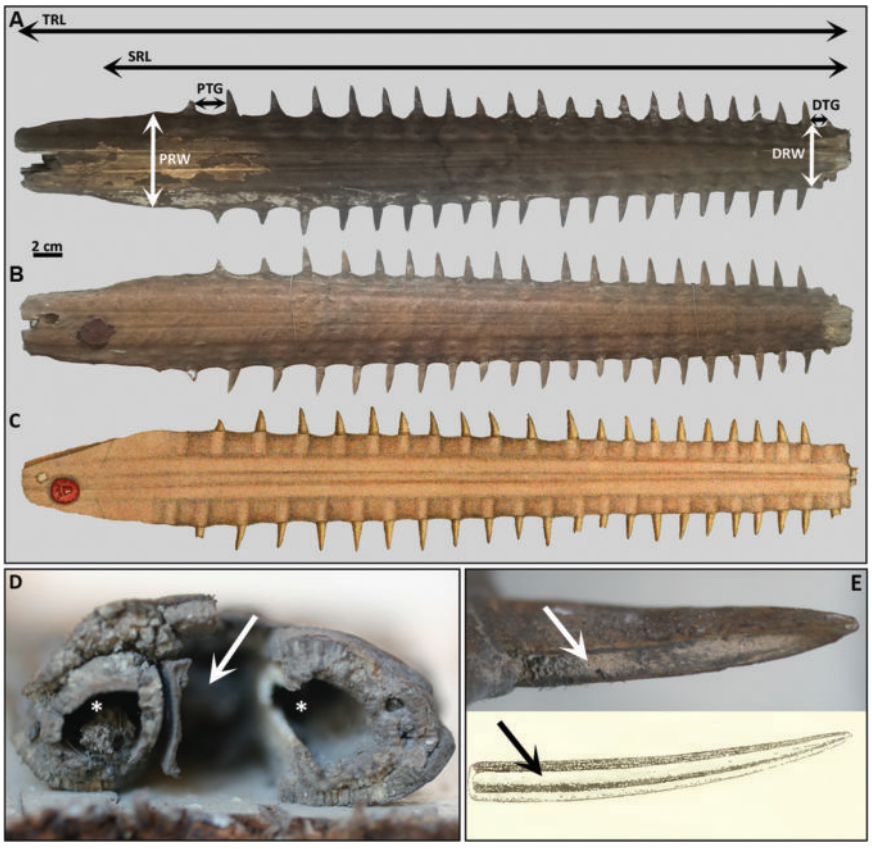

FIGURE 2 Morphological characters of the BSCM sawfish rostrum and comparison with illustrations from Costa (1853a, b): Pictures of the (A) dorsal and (B) ventral side of the sawfish rostrum showing the measurements carried out: total rostrum length (TRL), standard rostrum length (SRL), proximal rostrum width (PRW), distal rostrum width (DRW) proximal tooth gap (PTG) and distal tooth gap (DTG), and (C) illustration of the sawfish rostrum from Costa (1853b), (D) Picture of the proximal end of the rostrum with the three internal canals clearly visible, the median canal from which cartilage samples were taken is indicated by a white arrow and the two lateral canals are indicated with white asterisks, and (E) detail of a rostral tooth posterior margin compared to illustration from Costa (1853b); arrows indicate the groove.

each side and the first two pairs of basal-most teeth are missing. In light of the above limitations, the below measurements were taken as follows: standard rostrum length (SRL) = from mid-point of its distal-most tip to mid-point of the posterior edge of the proximal-most left rostral tooth alveolus; proximal rostrum width (PRW) = from the base of the second left rostral tooth alveolus to the opposite edge of the rostrum; distal rostrum width (DRW) = from the anterior edge of the 23rd left distal-most rostral tooth to the opposite edge; proximal tooth gap (PTG) = from the anterior edge of 3rd left tooth to the posterior edge of the 4th left tooth; distal tooth gap (DTG) = from the anterior edge of 23rd left tooth to the posterior edge of 24th left tooth; rostral narrowing (RN) = PRW/DRW.

Five morphological measurements (SRL, PRW, DRW, PTG, DTG), recognized as useful to assign dried rostra to sawfish species, were standardized as ratios (in mm) of TRL (in mm)

and multiplied by 100 to represent a percentage (Seitz & Hoover, 2017). The five standardized rostral characters were added to the dataset provided by Seitz & Hoover (2017), which includes measurements from 41 sawfish rostra belonging to individuals of *Anoxypristis cuspidata*, *P. pectinata*, *P. pristis* and *P. zijsron*, and a Principal Component Analysis (PCA) was performed using PAST 4.02 software (Hammer et al., 2001) to assign our rostrum to a sawfish species based on morphometric parameters.

Radiocarbon dating and histochemistry

To confirm the age of the rostrum, a radiocarbon dating analysis was carried out on a piece of cartilage tissue at the Centre for Dating and Diagnostics (CEDAD, University of Salento, Lecce, Italy) using the Accelerator Mass Spectrometry (AMS). The radiocarbon date was calibrated in calendar age using OxCal v4.4.4 software (Bronk Ramsey, 2021) and Marine13 calibration curve (Reimer et al., 2013). Calibration of radiocarbon dates obtained from marine samples is affected by the marine reservoir effect, a problem related to the fact that sea water is depleted in ^{14}C concentration compared to the atmosphere, bringing radiocarbon ages apparently older than the real ones. For this reason, the radiocarbon age needs to be calibrated using a global marine curve adequately corrected for local effects by applying a proper ΔR value for the geographical area of origin of the sample (Quarta et al., 2021). Due to doubts about the real geographical origin of the rostrum, the calibrated age was corrected for the local reservoir effect of different geographical areas from which the rostrum most likely originates: $\Delta R = 58 \pm 15\,^{14}C\,yr$ for the Mediterranean Sea (Reimer & McCormac, 2002), $\Delta R = 176 \pm 15\,^{14}C\,yr$ for (Ndeye et al., 2008) and a $\Delta R = 92 \pm 74\,^{14}C\,yr$ for Southwest Florida (Hadden & Schwadron, 2019).

The dry and brittle cartilage tissue of the fish was rehydrated prior to conducting histological analysis for evaluation of its conservation status. Briefly, the sample was immersed into a solution composed of 2–4% formaldehyde solution, pH 7.4, supplemented with 5% sodium carbonate. The sample was gently shaken for 12–16 hours to ensure optimal penetration of the solution into the material. After this period the solution was replaced by 4–6% formaldehyde, pH 7.4 without any further addition for another 12–16 hours (post-fixation) (Grove et al., 2015). Then the sample was decalcified in Osteodec solution (Bio-Optica) and it was subsequently processed for paraffin embedding and sectioned to a thickness of 3–5 μm. Sections were dewaxed in xylene and rehydrated through a graded series of ethanol. For histological examination, sections were stained with Safranin O and Gomori's paraldehyde fuchsin counterstained with Halmi's solution. Sections were examined with a light microscope Leica Leitz DMRBE (Leica Microsystem) equipped with a digital analyzer (Leica LAS 4.0).

Genetic analysis

DNA extraction and Polymerase Chain Reaction (PCR) setup were performed in a facility dedicated to the analysis of ancient DNA (aDNA) following stringent precautions to avoid contamination by exogenous DNA (Cooper & Poinar, 2000; Pääbo et al., 2004; Knapp et al., 2012). The aDNA facility was located in a building far from the one where the post-PCR phases occurred and was regularly decontaminated using bleach or LookOut® DNA Erase (Sigma-Aldrich) and UV irradiation (254 nm). Only authorized personnel equipped with protective devices (disposable body suits, dedicated footwear, gloves, face mask, laboratory goggles) had access to the facility. In addition, potential contamination

was monitored by processing negative controls with the sample during both the DNA extraction and PCR setup phases. DNA of the sawfish rostrum under investigation was extracted from a small piece of cartilaginous tissue sampled from the median canal of the rostrum, the sampling area was selected so as not to damage the important relic externally. The cartilage tissue was decontaminated and subjected to DNA extraction following Fioravanti et al. (2020a), and then small fragments of the mitochondrial DNA (mtDNA) were amplified to identify the rostrum at the species level. A 187 bp fragment of the cytochrome c oxidase subunit I (COI) gene was amplified using a single primer pair (PpCOI_F: 5'-CCTCCATCATTCCTCCTACTG-3', PpCOI_R: 5'-TTGTTGTAATAAAGTTAATGGATGCT-3') and a 324 bp fragment of the NADH dehydrogenase subunit 2 (ND2) gene was amplified using two primer pairs (PpND2_F: 5'-CCCCAAACATGTTGGTTAAAA-3', PpND2_R: 5'-GGCTTCTACTGCTCGTGGAT-3', PpND2b_F: 5'-ACCATAGCCATCATCCCATT-3', PpND2b_R: 5'-GTGTGGCAGAGACTGGGTTT-3'). Primer pairs were designed using Primer3Plus software (Untergasser et al., 2012) based on the mtDNA complete genome of *P. pectinata* available in GenBank (Accession number: NC_027182). PCR amplification was carried out in a reaction volume of 25 µl containing 2.5 µl of 10X PCR buffer; 0.5 µL of 10 µM each dNTP (Invitrogen), 0.75 µL of 50 mM $MgCl_2$, 2 µL of 5µM primer mix, 0.125 µL of 20 mg/ml Bovine Serum Albumin (BSA, Thermo Fisher Scientific), 0.2 µL of Platinum™ Taq DNA Polymerase (Invitrogen), 5 µl of DNA and 13.925 µL of ultrapure sterile water. The amplification was performed on a T100 Thermal Cycler (Bio-Rad) dedicated to working on aDNA and programmed as follows: an initial denaturation at 94°C for 2 min, 60 cycles of denaturation at 94°C for 30 s, annealing at 54°C for 30 s, extension at 72°C for 30 s and a final extension at 72°C for 7 min. All PCR products were visualized on a 2% agarose gel stained with GelRed® (Biotium) and, after purification by ExoSAP-IT™ (Applied Biosystems), they were Sanger sequenced on an ABI PRISM 3730xl DNA Analyzer (Applied Biosystems) at the BMR Genomics (Padova, Italy). All sequences obtained were compared by nucleotide BLAST (Altschul et al., 1990) with those available in the GenBank database to allow species identification. COI and ND2 sequences for all the sawfish species described so far were extrapolated from mtDNA reference sequences (GenBank accession numbers: *A. cuspidata*, NC_026307; *P. pectinata*, NC_027182; *P. pristis*, NC_039438; *P. zijsron*, MH005927; *P. clavata*, NC_039438) and aligned using CLUSTALW (Larkin et al., 2007) with those obtained from our specimen. In addition, the ND2 sequence was also aligned with ND2 haplotypes detected for *P. pectinata* (Faria et al., 2013) to assign a specific haplotype to our sample and try to identify the most probable geographical origin of the sawfish rostrum exposed at the BSCM. To highlight evolutionary relationships among sawfish species, COI and ND2 sequences were combined, aligned and used to construct an unrooted phylogenetic tree. A neighbor-joining tree analysis (Saitou & Nei, 1987) was performed on MEGA v11 software (Tamura et al., 2021) using the estimated best nucleotide substitution model TN93+G (Tamura & Nei, 1993), with 1,000 bootstrap replicates and considering pairwise deletion in case of ambiguous sites.

Results

Morphology and meristics

The BSCM rostrum appears relatively narrow and its sides moderately tapering distally (fig.

2A, B, C). The rostrum-head juncture is not visible and three internal canals are identifiable in cross-section at the level of the basal portion of the rostrum and its broken tip (fig. 2D). Measurements and counts carried out in this study are shown in table 1, together with those published by Costa (1853b) on the same rostrum and by Seitz & Hoover (2017) on isolated rostra of *P. pectinata* individuals. The estimated TL of the sawfish specimen is ca. 3010 mm and TRL is 770 mm, so the rostrum is 25.6% of the estimated TL. Visible rostral teeth (including alveoli lacking teeth) are 24 on the left and 23 on the right side. The rostrum is cut on each side at the level of the first two pairs of basal-most teeth and it is broken at the tip where probably one additional tooth per side was present, making the total rostral tooth count equal to 49 (25 on the left and 24 on the right side) (figs. 2A, B, C). The alveoli of the first and second pair of rostral teeth, as well as the alveolus of the 23rd rostral tooth on the right side, are well visible. Rostral teeth are present along the entire rostrum, including the basal quarter of the saw. They are moderately flattened, enlarged and awl-like with rounded anterior and, flattened and grooved posterior margins. The groove at the posterior margin of the rostral teeth reaches its base (fig. 2E). The longest tooth is about one-third of PRW; proximal tooth gap (PTG) ca. 44% of distal tooth gap (DTG); proximal rostrum width (PRW) ca. 1.5 times of distal rostrum width (DRW); proximal tooth gap (PTG) ca. 35.5% of proximal rostrum width (PRW). PCA performed on five standardized rostral characters (SRL, PRW, DRW, PTG, DTG) allows to separate rostra belonging to different sawfish species as demonstrated by Seitz & Hoover (2017). The rostrum of the BSCM plotted closer to the *P. pectinata* polygon area (see fig. 3) thus supporting the taxonomic determination obtained through our classical morphological study.

Radiocarbon dating and histochemistry

The conventional radiocarbon date estimated for the sawfish rostrum of the BSCM is 407 ± 35 BP, corresponding to a calibrated age range of cal AD 1851–1953 (95.4% probability) considering the Mediterranean reservoir effect, cal AD 1890–1968 (95.4% probability) considering the Senegal reservoir effect and cal AD 1801–1953 (89.7% probability) considering the Southwest Florida reservoir effect.

Histochemical staining carried on cartilage tissue with Safranin O shows large areas of metachromasia (orange stain) which demonstrate the presence of sulphated glycosaminoglycans with a typical cartilaginous appearance (fig. 4A). Numerous rounded cells are homogenously distributed in all the sample. These cells show a chondral-like appearance being located in their lacunae and surrounded by a metachromatic intercellular matrix (orange stain). Several chondrocytes show a preserved nucleus while some lacunae appear empty (figs. 4A, B). Gomori's fuchsin paraldehyde/Halmi method that stains the connective tissue matrix in red and green, showed no fibroblast-like flattened cells or elastic fibres, suggesting that this tissue is mainly composed of hyaline rather than fibrous or elastic cartilage (fig. 4B).

Genetic analysis

Genetic analysis performed on both COI and ND2 fragments confirmed the morphological identification of the sawfish rostrum as belonging to a *P. pectinata* individual. The BLAST search revealed a 100% identity with *P. pectinata* sequences available in GenBank, as also demonstrated by the alignment among reference sequences of *A. cuspidata*, *P. pectinata*, *P. pristis*, *P. zijsron* and *P. clavata* (fig. 5A, B). In addition, the neighbor-joining tree constructed using a combination of COI and ND2 sequences graphically shows that the BSCM rostrum groups well with *P. pectinata* (fig. 5C).

TABLE 1 Measurements and counts of the BSCM sawfish rostrum compared with measurements and counts published in Costa (1853b). The range of values (as % of TL), and mean values (±SD) included in brackets for *P. pectinata* provided by Seitz & Hoover (2017) are also presented

Measurements (mm)	**BSCM (present study)**	**as % of TRL**	**BSCM (Costa, 1853b)**	**as % of TRL**	**Seitz & Hoover, 2017**
Total rostrum length (TRL)	770		785		176–835 (549.7 ± 258.1)
Standard rostrum length (SRL)	700	90.9	N.A.		92.4–96.5 (94.7 ± 1.6)
Proximal rostrum width (PRW)	90	11.7	88	11.2	12.4–17.3 (14.6 ± 1.6)
Distal rostrum width (DRW)	56	7.3	54	6.9	5.4–8.5 (7.1 ± 1.1)
Proximal tooth gap (PTG)	32	4.2	31	3.9	3.2–6.9 (5.4 ± 1.0)
Distal tooth gap (DTG)	14	1.8	18	2.3	1.1–2.5 (1.9 ± 0.4)
Longest tooth	29	3.8	29	3.7	N.A.
Shortest tooth	21	2.7	27	3.4	N.A.
Rostral narrowing (PRW/DRW)	1.6		1.7		1.8–2.3 (2.1 ± 0.2)
Teeth Count					
Number of rostral teeth on left side	24*		24		21–27 (24.8 ± 1.9)
Number of rostral teeth on right side	23**		24		21–28 (25.1 ± 2.0)

Abbreviations and symbols:
*, possibly 25 considering the broken tip;
**, possibly 24 considering the broken tip; N.A., not available

Comparing the diagnostic nucleotide positions identified by Faria et al. (2013) aligning different ND2 haplotypes of *P. pectinata*, the ND2 sequence obtained from the rostrum was recognized as haplotype "C" (fig. 5B) previously found in samples from the western and eastern Atlantic Ocean (see Faria et al., 2013). Sequences of the CO1 and ND2 obtained from the BSCM sawfish rostrum here analyzed were uploaded in GenBank under accession numbers OQ547785 and OQ547786, respectively.

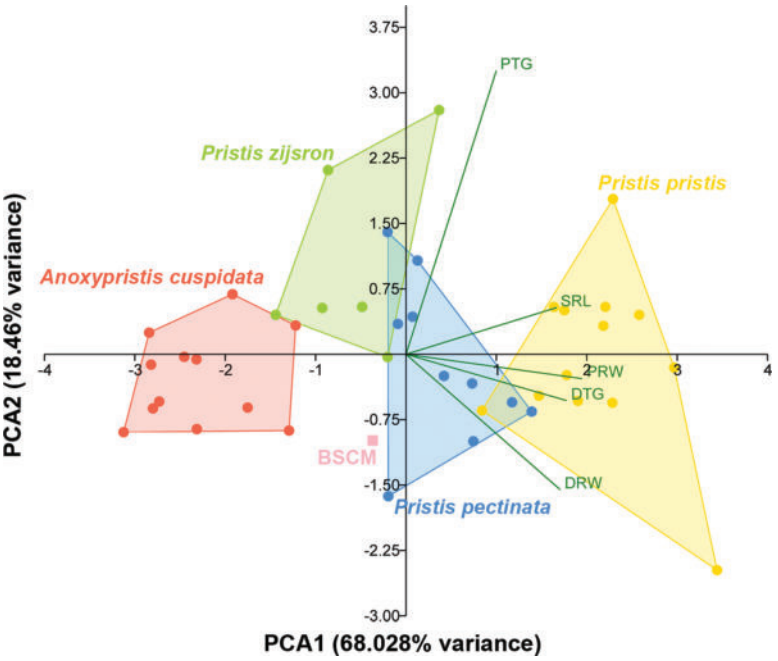

FIGURE 3 Principal component analysis (PCA) performed on five standardized rostral characters (SRL, PRW, DRW, PTG, DTG) using measures obtained from the rostrum of the BSCM (pink square) and data from Seitz & Hoover (2017) for isolated rostra of *A. cuspidata* (in red), *P. pristis* (in yellow), *P. pectinata* (in blue) and *P. zijsron* (in green). Each rostrum is indicated with a dot, while groups of rostra of the same species are represented by a polygon.

Discussion

Remarks on rostrum history and age

The first mention of the presence of the sawfish rostrum in the BSCM dates back to 1853, when Carmelite Fathers, in charge of the basilica, published an anonymous booklet with the aim to determine the species to which the rostrum belonged. The rostrum description and taxonomic determination were entrusted to Oronzio Gabriele Costa, an eminent Neapolitan zoologist, on the occasion of the visit of the King Maximilian II of Bavaria. The king was an amateur naturalist and, to celebrate his visit to Naples, the story was published and donated to the sovereign (Costa, 1853a). A detailed morphological description of the rostrum was included in the booklet by Costa who attributed it to an individual of *P. antiquorum* Latham, 1794, a nominal species that is currently a synonym of *P. pristis*. In addition, the rostrum was dated back to 1573 and originating from the Mediterranean Sea, as it was recognized as belonging to a fish involved in a "miraculous event" described by Tommaso Costo (1588), a Neapolitan hagiographer. The miracle occurred in 1573 and involved a Spanish galleon that left Naples for a military expedition to Tunis, during the war between the Kingdom of Spain and the Ottoman Empire for control of the western Mediterranean. The galleon risked sinking due to a leak in the hull and, according to the original text, an unidentified fish entered through the leak and plugged it, thus preventing the ship from sinking. In memory of the "grace"

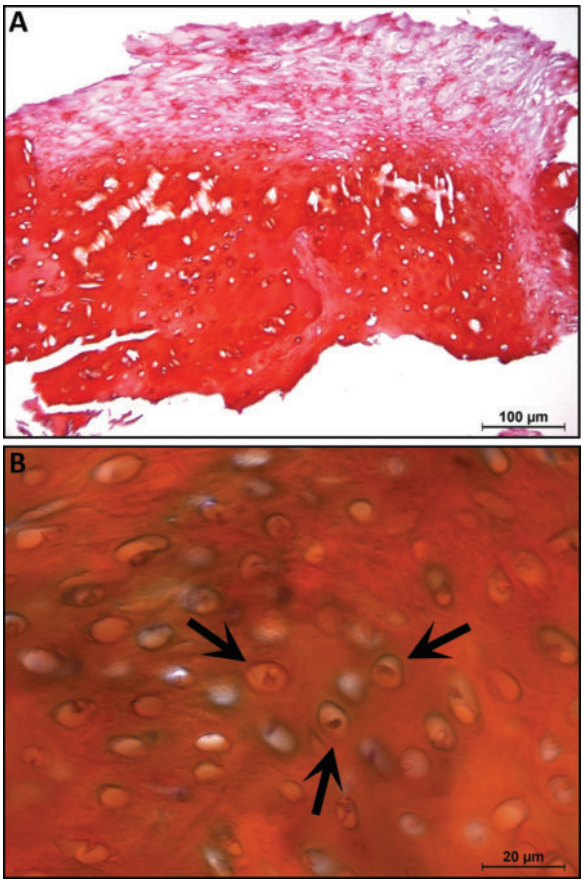

FIGURE 4
Safranin O staining method (A) highlights large areas of metachromasia (orange stain), indicating the presence of sulphated glycosaminoglycans, surrounds empty lacunae and lacunae containing chondrocytes with preserved nucleus. Gomori's fuchsin paraldehyde/Halmi method (B) stains cellular nuclei in dark violet and connective tissue matrix in red and green. Some cells containing visible nuclei are indicated by black arrows.

received from "Our Lady of Mount Carmel", to whom the crew members appealed in time of need, a model of the galleon was donated to the BSCM as an *ex voto*. Later, Bacco (1605) published a version of the text that was essentially identical but added that the model of the galleon included the fish in the leak (fig. 1B).

The taxonomic classification of the rostrum and, information about age and geographical origin deduced from the story of the "miraculous event" led some authors to claim the sawfish rostrum of the BSCM as the most ancient example of largetooth sawfish belonging to the Mediterranean population (Ferretti, 2014; Ferretti et al., 2016) however, radiocarbon dating performed in our study shows that the sawfish rostrum is more recent than claimed. Considering the local Mediterranean marine reservoir effect, the calibrated age is cal AD 1851–1953, an age closer to the date of publication of Costa's description (1853a, b) rather than to that reported in the story of the "miraculous event" (1573). Statistical errors in the radiocarbon dating of marine samples can be frequent if the marine reservoir effect is not corrected using appropriate ΔR values for the geographical origin of the sample (Quarta et al., 2021), in this case, a wrong radiocarbon date could be obtained if the rostrum did not come from a Mediterranean sawfish. The smalltooth sawfish is restricted to the Atlantic Ocean (Faria et al., 2013); along the western Atlantic coast a major distribution is currently recorded in southwest Florida (Brame et al., 2019) while, in the eastern Atlantic it has been extirpated from much of its former range

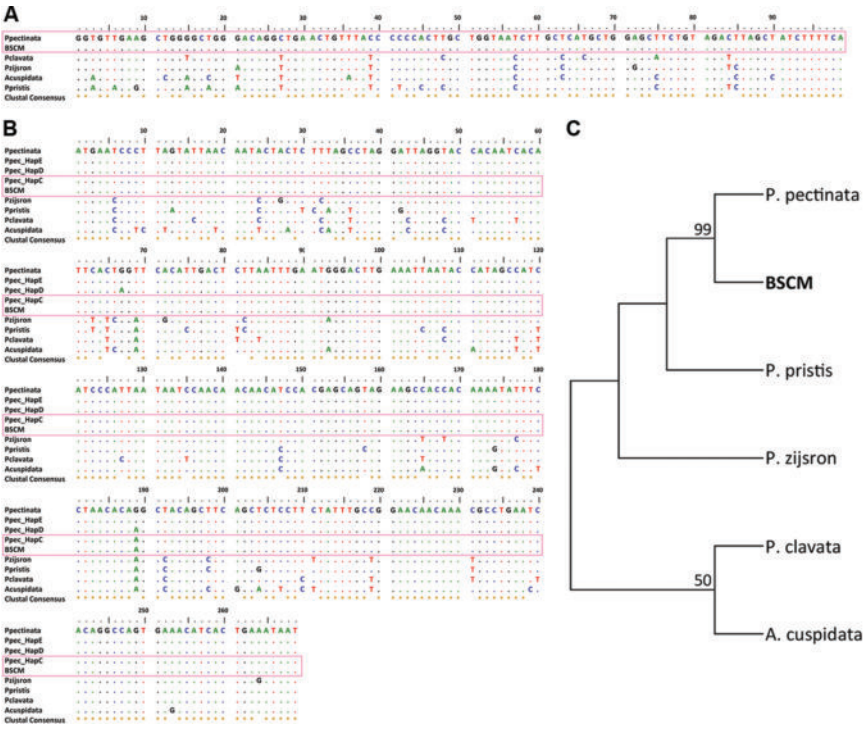

FIGURE 5 Alignment of COI (A) and ND2 (B) sequences obtained for the rostrum of the BSCM (indicated by a pink box) and those available in GenBank for sawfish species (see main text for details and Accession numbers). ND2 haplotypes for *P. pectinata* from Faria et al. (2013) are indicated as HapC, HapD, HapE. Unrooted neighbor-joining tree (C) constructed using COI + ND2 sequences and displaying only >50% percentage bootstrap scores.

(Robillard & Séret, 2006; Leeney & Downing, 2016; Downing & Leeney, 2019). Considering the species distribution, the conventional radiocarbon date obtained for the BSCM rostrum was also calibrated using the ΔR values for Senegal and southwestern Florida to highlight any discrepancies between obtained ages. In the first case the calibrated age is cal AD 1890–1968 and, in the second case is cal AD 1801–1953. These results demonstrate that the rostrum would not be older than 200 years even if it comes from the Atlantic Ocean, suggesting that information on the age and origin of the rostrum deduced from the legend are most likely incorrect. In this regard, it is noteworthy that Costa himself (1853a) marvels that a specimen of such interest had so far been ignored by the Neapolitan zoologists. In the light of our results, this appears less strange because, evidently, before the rostrum was simply not present inside the church.

In our opinion, the legend about the sawfish rostrum kept in the BSCM is most likely related to the symbolic significance of the sawfish in the Middle age rather than to a real event, and closely related to the tradition of displaying in convents and churches sawfish rostra donated by believers as "votive offerings" (Márquez-Rodríguez, 2014). Carmelite Fathers probably decided to prepare a reliquary with a real sawfish rostrum, on the occasion of the visit of the King of Bavaria, to enrich the story of the "miraculous event". The story originates in the period of extreme contrast between the Islamic and Christian worlds, resulting in the battle of Lepanto in 1571 that ended with

the victory of the Christian fleet of the Holy League under the insignia of the Virgin Mary, and its creation is consistent with the historical and cultural climate spread in Italy during the period of the Catholic Reformation. All elements of the story have a strong symbolic significance perfectly consistent with that mentioned in the ancient Christian text "Physiologus" (3rd or 4th century AD; Scott, 1998), deeply imbued with Christian symbology (Zambon, 2018). The sawfish is here depicted as a diabolic animal that competes with ships, representing the Church, and then sinks them into the deep sea, alluding to the depths of Hell. In this description, the sailors symbolize the Christian people tempted by the lure of the fish which is however defeated by divine providence. This allegory is consistent with the Neapolitan legend, with the variant that the sawfish is used by the Virgin Mary to save sailors. Our interpretation is therefore reinforced by the ancient medieval custom of displaying crocodiles, animals considered symbols of the devil, inside Marian churches as a sign of submission of the devil to the divine majesty through the intercession of the Virgin Mary (e.g., Bertelli, 2018; Fioravanti et al., 2020b). The non-Neapolitan origin of the rostrum is also suggested by the original lacquer wax stamp attached to the ventral part of the rostrum which is also shown in Costa's illustrations (1853a, b). This stamp corresponds to the logo of the Roman Curia of the Carmelite Order with which "votive offerings" from the headquarters in Rome were marked, while a "votive offering" owned by the BSCM would have been marked with a different stamp bearing the logo of the Carmelite fathers of Naples (M. Rea, pers. comm.; fig. 1C).

Taxonomical and phylogeographical remarks

Morphological analysis was performed in this study to validate the taxonomic identification by Costa (1853a, b) of the species to which the rostrum kept in the BSCM belongs. Except for the distal tooth gap (1.4 vs 1.8 cm) and shortest tooth (2.1 vs 2.7 cm), our measurements and counts obtained from the sawfish rostrum agree quite well with the data published by Costa (1853b). The rostrum total length was slightly smaller with respect to Costa's measure (77 vs 78.5 cm), whereas the proximal rostral width (9 vs 8.8 cm), distal rostral width (5.6 vs 5.4 cm), and proximal tooth gap (3.2 vs 3.1 cm) were slightly larger. Differences between our measurements and the ones published in Costa (1853b) are probably due to different methodologies in taking measurements and/or defective conversion of measuring units (Neapolitan palms and Parisian thumbs) used by the latter author. As correctly noted by Costa (1853a), the sawfish rostrum exhibits a bilateral asymmetry in the rostral teeth position. However, in contradiction with the above statement, Costa erroneously counts 24 teeth per side (including two pairs of empty alveoli basally). Our assumption is that the missing tip of the rostrum contains one additional tooth per side making the total rostral tooth count equal to 49 (25 on the left and 24 on the right side). Worthy of note, is that the figures of the BSCM sawfish rostrum illustrated by Costa (1853a, b) show the ventral instead of the dorsal side (fig. 2C) as proven by the difference in pigmentation between the dorsal (darker) and ventral (paler) sides (fig. 2A, B). This mistake may be explained by the fact that the rostrum is mounted upside down on a wooden stand within its glass display case (fig. 1A). Costa was probably unaware of the original wrong positioning of the rostrum within its display case and reproduced it exactly as he found it. In addition to the rostrum, Costa (1853a, b) illustrates a full-size tooth in the dorsal and latero-posterior view. The groove at the posterior margin clearly extends to the tooth base, a condition present

in all the teeth as attested by our study (fig. 2E). The latter characteristic, in combination with other morphological features, is commonly used to distinguish among different sawfish species (Faria et al., 2013), including isolated rostra (Whitty et al., 2014).

As pointed out by Faria et al. (2013), the original description of *P. pristis* (Linnaeus, 1758) most probably represents a "composite species", i.e., based on specimens of both *P. pristis* and *P. pectinata*. Moreover, the poor descriptions and citations referring to different sawfish species included in Latham's (1794) revision, made it tricky for later authors to interpret and identify *P. antiquorum* as a large-tooth sawfish. Therefore, it is not surprising that Costa misidentified the sawfish rostrum of the BSCM as belonging to an individual of *P. antiquorum* Latham, 1794, a nominal species presently considered to be a synonym of *P. pristis*. Our measurements and counts made on the sawfish rostrum of the BSCM agree well with data from isolated rostra published for *P. pectinata* (Seitz & Hoover, 2017). The slight differences in SRL, PRW, and PRW/DRW ratios of the BSCM rostrum with respect to values published by the latter authors, probably reflect the condition of the rostrum (i.e., broken tip, cuts on the sides of the basal portion, etc.) and/or individual or geographical variation. All other ratios (DRW, PTG, DTG) and tooth counts fall well between the ranges provided by Seitz & Hoover (2017) for *P. pectinata* (see table 1). When data obtained from our rostrum were combined with those from Seitz & Hoover (2017) and plotted using the PCA method, the identification of the rostrum as *P. pectinata* using morphometric parameters becomes evident (see fig. 3).

DNA extracted from historical samples is usually available in small amounts and highly degraded, characteristics which limit the efficiency of the PCR amplification. In addition, the possible contamination by exogenous DNA could complicate the interpretation of genetic results (Raxworthy & Smith, 2021). The possibility to successfully obtain non-degraded or partly degraded DNA from the skin of dry sawfish rostra was demonstrated for the first time by Phillips et al. (2009), thus paving the way for the use of rostra from museums and/or private collections for conservation genetic studies of these endangered species. Histochemical methods applied in this study demonstrated that the tissue consists exclusively of well-preserved hyaline cartilage which seems particularly suitable for DNA extraction. Staining methods highlight the presence of several chondrocytes containing nuclei clearly visible, and the subsequent genetic analysis allowed us to obtain good quality amplicons and sequences for both mtDNA markers chosen for genetic analysis. The hyaline cartilage from the median canal of the rostrum is therefore a useful tissue, together with skin (Phillips et al., 2009), for the extraction of good quality DNA for the identification of sawfish rostra. In addition, the sampling of hyaline cartilage is completely invisible and does not involve an invasive procedure that usually holds back museum curators from approving the sampling (Raxworthy & Smith, 2021). All sawfish species, except *A. cuspidata,* have a rostrum with three internal canals running from the head of the sawfish to the distal end of the rostrum (Wueringer et al., 2009; Whitty et al., 2014; Byler, 2017), the median canal effectively contains hyaline cartilage while the two lateral canals host the rostral artery and ophthalmic and buccal nerves (Wueringer et al., 2009; Byler, 2017). The surface of the rostrum and lateral canals are additionally covered by calcified cartilage which gives rigidity and strength to the rostrum (Wueringer et al., 2009; Byler, 2017). The hyaline cartilage sampled from the sawfish rostrum of the BSCM may have been well preserved as it is

protected from microorganisms and environmental factors that affect the degree of conservation of both cartilage tissue and DNA (Byler, 2017). Furthermore, the storage of the rostrum in a glass display case could have protected it from environmental and biological agents, the main causes of DNA degradation in historical samples.

Two mtDNA molecular markers, COI and ND2 genes were chosen for genetic analysis as they proved to be useful for the: i) identification of sawfish species involved in the illegal fin trade of elasmobranchs (Rodrigues Filho et al., 2020); ii) evaluation of the presence in some geographical areas of different sawfish species by the analysis of environmental DNA (Simpfendorfer et al., 2016; Lehman et al., 2020); and iii) especially for the description of isolated rostra from natural history collections (Faria et al., 2013). Both COI and ND2 fragments were successfully amplified and sequenced showing the good quality of DNA extracted from hyaline cartilage. Furthermore, the alignment highlights that the BSCM sawfish rostrum belongs, without any doubt, to *P. pectinata* rather than to *P. pristis* as erroneously reported by some contemporary authors (Ferretti, 2014; Ferretti et al., 2016; Bargnesi et al., 2020). Earlier authors, like Tortonese (1956) and Bini (1967), followed Costa's (1853a, b) taxonomic determination (i.e., *P. antiquorum* Latham, 1794) of the BSCM rostrum and included it among the list of synonyms of *P. microdon* Latham, 1794, a nominal species presently considered a synonym of *P. pristis*. Subsequently, Tortonese (1982, 1985, 1987) in broader scope papers dealing with the Mediterranean ichthyofauna, neglected the presence of sawfishes in the Mediterranean Sea.

The geographical origin of the BSCM sawfish rostrum cannot be definitively clarified even using the results of genetic and morphological analyses. The alignment of the ND2 sequence obtained from our sample with those of the three haplotypes previously described for *P. pectinata* (Faria et al., 2013) revealed that the sawfish rostrum belongs to the haplotype "C". The haplotype "C" was found in individuals from the western Atlantic and also from the eastern Atlantic, as demonstrated by the analysis of two museum specimens from Senegal and Congo, indicating the lack of geographical structure among western and eastern Atlantic populations (Faria et al., 2013). On the other hand, morphological analysis indicates that a population structure among the two sides of the Atlantic Ocean could be observed based on rostral tooth count variation (Faria et al., 2013; Downing & Leeney, 2019). With a mean number of 23.5 (possibly 24.5) rostral teeth per side, the BSCM rostrum shows a closer affinity to the eastern Atlantic (23.1 from Downing & Leeney, 2019) rather than to the western Atlantic (25.4 from Downing & Leeney, 2019) population, suggesting a possible West African origin of the sample. Conversely, the bilateral asymmetry displayed by the BSCM rostrum does not fit well with the hypothesis of a West African origin. While bilateral asymmetry seems to be a common attribute of *P. pectinata* populations in the western Atlantic (Wiley et al., 2008), the proportion of individuals displaying bilateral asymmetry in a smalltooth sawfish population in Senegal (eastern Atlantic) was not significantly greater than those that displayed bilateral symmetry (Downing & Leeney, 2019) suggesting that the BSCM rostrum probably had a mean number of 24.5 (instead of 23.5) rostral teeth per side in the undamaged condition and that it most probably originated from the western Atlantic. An American origin was also proposed for the *P. pectinata* rostrum kept in the church of Santa María de la Mota (Marchena, Spain) based on the constant trade between America and Spain after Columbus' expeditions and on the presence of other sawfish rostra in Spanish

churches and museums with an American origin (Márquez-Rodríguez, 2014).

Conclusions

Morphological and genetic analyses performed in this study clearly show that the BSCM sawfish rostrum belongs to a *P. pectinata* rather than to *P. pristis*, as stated previously by other authors (Tortonese, 1956; Bini, 1967; Ferretti, 2014; Ferretti et al., 2016; Bargnesi et al., 2020). All these authors took for granted Costa's determination of the sawfish rostrum (Costa, 1853a, b) thus leading to a circular process of misidentification interrupted only now thanks to our study. This unfortunate example of taxonomic confusion demonstrates the importance of including taxonomy experts in historical ecology works and it also shows that classical morphological methods and molecular barcoding are complementary approaches that must be used together to improve taxonomic and phylogenetic inferences. In addition, the estimated radiocarbon age, and the impossibility to confirm the Mediterranean origin of the rostrum highlights that information concerning ancient and historical samples is not always correct and that further analyses must be performed to estimate the age and origin of samples when they are used to detect changes in natural populations or to reconstruct the evolutionary history of species. While our study is not aimed at tackling the debated issue dealing with the presence or absence of resident, breeding sawfish populations in the Mediterranean Sea, it demonstrates that historical accounts of sawfish records should be critically assessed before using them to draw any far-reaching conclusion on questions dealing with their historical ecology and/or biogeography, and that future studies using historical information and specimens should adopt a similar integrative taxonomy approach as used in our study. Finally, the application of modern genetic techniques to the analysis of ancient and historical samples allows to give a "second life" to museum and archaeological samples as demonstrated by the fundamental work of Svante Pääbo, Nobel Prize for Medicine in 2022.

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Carmine di Napoli. Con alcune gratie fatte da essa Madonna del Carmine à suoi divoti. Raccolta brevemente da altri Autori, e dalle memorie antiche della sua Chiesa, da Henrico Bacco. Con Privilegio. Per Costantino Vitale, Napoli.

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