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## Updating knowledge on the Critically Endangered white skate *Rostroraja alba* (Lacepède, 1803) through the first record in North-East Sardinian coastal waters

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### Abstract

The white skate *Rostroraja alba* has exhibited a declining trend similar to that of several large-bodied batoids in recent years. Consequently, it has been classified as Critically Endangered in European waters by the International Union for the Conservation of Nature. The presence of this species in Sardinian seas (central-western Mediterranean Sea) was recently hypothesised following the discovery of a hatched eggcase. Our findings confirmed this hypothesis through the discovery of another eggcase (this time unhatched) and, more importantly, the capture of an immature female (total length 131.2 cm) which was estimated to be 6 years old. Moreover, molecular analysis demonstrated that the new eggcase and the specimen did not share the same mother, thus suggesting the presence of a resident population, albeit likely small, of the species in the Sardinian seas.

**Keywords:** Mediterranean Sea; COI and NADH2; Age and growth; Elasmobranchs; Distribution Areas.

### Introduction

Cartilaginous fish are widely acknowledged as one of the most vulnerable vertebrate groups to the impacts of human activity (O’Keefe *et al.*, 2023). Currently, the International Union for the Conservation of Nature considers more than one-third of elasmobranch populations worldwide to be at risk of extinction due to a sharp decline. (IUCN, 2023). This decline has numerous causes, all of which are human-induced causes, such as habitat deterioration due to pollution, either directly (in the ecosystem) or as the primary source of climate change. Nonetheless, out of all the major causes of human disturbance, overfishing, with elasmobranchs being both targeted and constituting by-catch or even discards, has been indicated as the most impacting (Dulvy *et al.*, 2021). This circumstance is often linked to the distinctive life-history traits typical of elasmobranchs, which are frequently identified as slow-growing species with late maturity (Maioli *et al.*, 2023). However, not all elasmobranch species seem to be equally affected. For example, small-bodied skates, which usually tend to grow faster compared to other batoids (e.g., Porcu *et al.*, 2020; Vil-

lagra *et al.*, 2022), appear to suffer less the consequences of overfishing, sometimes even showing an increase in their population by occupying ecological niches left empty by the disappearance of large-sized skates, which are more severely impacted by overfishing (Maioli *et al.*, 2023). Large skates are thus facing a severe reduction in numbers globally, with the extreme result of the almost complete depletion of their populations in some parts of their habitats. For instance, some of the largest skate species [e.g., *Dipturus* sp. and the white skate *Rostroraja alba* (Lacépède, 1803)], inhabiting the Northeast Atlantic Ocean and Mediterranean Sea are showing a dramatic decline in recent decades, even being considered as locally extinct in some areas (Ramírez-Amaro *et al.*, 2020; Melis *et al.*, 2020 and reference therein).

The white skate *R. alba* is a large-bodied skate distributed in the Eastern Atlantic Ocean including the entire South African coast and the Mediterranean Sea (Dulvy *et al.*, 2006; Last *et al.*, 2016), where it is however considered rare (Serena *et al.*, 2010) and probably subject to a sharp decline in abundance (Ellis *et al.*, 2015). In the Mediterranean Sea, the species has been recorded along the Moroccan and Tunisian coasts, while in the Europe-

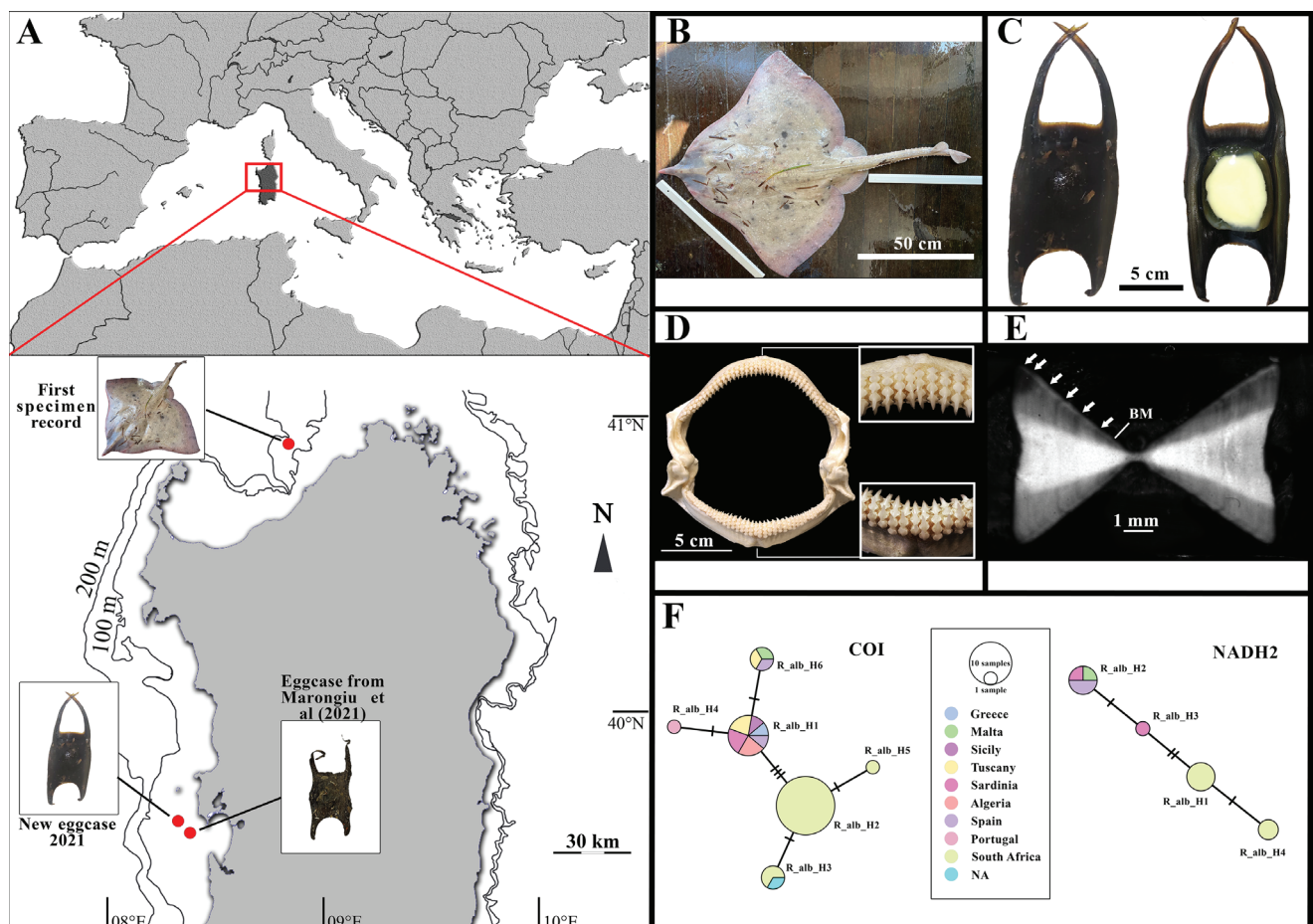
an side of the basin, *R. alba* has been registered in the Balearic Islands, Corsica, the Strait of Sicily and the North Adriatic Sea (Follesa *et al.*, 2019a). According to Dulvy *et al.* (2006), the species could have a wider geographical range, reaching Greece and Turkey in the Mediterranean Sea and Ireland in the North-East Atlantic Ocean, and extending into the southwestern parts of the Indian Ocean. Nonetheless, some areas have no valid records and the geographic range of this species is still hardly discussed (Dulvy *et al.*, 2006). Recently, the presence of the species has been hypothesised in Sardinian waters (central-western Mediterranean Sea) following the discovery of an already hatched eggcase (Marongiu *et al.*, 2021). However, while this offers a strong hint, the discovery of a single hatched eggcase might not definitively prove the species' presence in the area, as due to its very low weight it could have been easily carried out by the currents. Furthermore, this could be especially true considering that the eggcase from Marongiu *et al.* (2021) appeared to have hatched quite some time before its discovery, as it was also covered by well-grown serpulid tubes.

In consideration of the above, and taking advantage of the first catch of a white skate specimen, together with the finding of a second eggcase, this time unhatched, the present paper aims to confirm the presence of the white

skate in Sardinian seas. We also provide the first biological information regarding this rare and endangered species (listed in the IUCN Red Lists as Endangered and Critically Endangered at Global and European levels, respectively; Ellis *et al.*, 2015; Serena *et al.*, 2024), including the production of the first molecular sequences from individuals in the area.

## Material and Methods

Both the new *R. alba* eggcase and the specimen were found during the MEDITS (MEDiterranean International Trawl Survey) scientific survey (Spedicato *et al.*, 2019) conducted in the FAO-GFCM Geographic Sub-Area (GSA) 11 – Sardinian seas (Fig. 1A, B). The eggcase was photographed with a high-resolution digital camera (Canon 1100D), including a scale (Fig. 1C). From the obtained images, the principal morphometric measurements for elasmobranchs' eggcases were recorded following Concha *et al.* (2012) and Porcu *et al.* (2017). Similarly, the specimen was photographed with the same equipment, and the principal biometric measurements were annotated from the images following McEachran & Compagno (1982). Additionally, the total mass (TM) was recorded,



**Fig. 1:** Geographical map representing the Mediterranean Sea and displaying the recent finding of *R. alba* specimen, new eggcase and including the last record from Marongiu *et al.* (2021) (A); picture showing the individual caught in 2023 (B); image of new eggcase showing dorsal and opened ventral view (C); picture of specimen' jaws with details of upper and lower arches (D); vertebral section of *R. alba* specimen with white arrows indicating translucent bands and BM = birthmark (E); graphical representation of TCS haplotype networks with each bar representing a mutation event (F).

and the sexual maturity stage was macroscopically estimated according to Follesa *et al.* (2019b). Finally, a portion of the vertebral column was extracted from above the abdominal cavity and preserved frozen according to Kadri *et al.* (2014). Subsequently, to estimate the specimen's age, vertebral centra were cleaned, sectioned, and analysed following Porcu *et al.* (2020) (and references therein).

Total genomic DNA (gDNA) was extracted following the Salting Out extraction protocol (Miller *et al.*, 1988) from the two new Mediterranean specimens (i.e., eggcase and individual) and from four South African specimens. PCR reactions were performed in 25 µl total volume containing 1x PCR buffer (MgCl and dNTPs included), 2.0 pmol of each primer, and 1.25 U of WonderTaq Hot Start DNA polymerase (EuroClone). The amplifications were performed in a Mastercycler EP Gradient S Eppendorf (Biometra). A fragment of the mitochondrial gene COI was obtained from each specimen by PCR using the FishF2 and FishR2 primers (Ward *et al.*, 2005). PCR reactions were performed with an initial denaturation of 3 minutes at 95° C, followed by 35 cycles of 30s at 95° C, 30s at 55° C and 60s at 72° C and a final extension step for 5 minutes at 72° C. Similarly, a fragment of the NADH2 gene was amplified for all individuals using the ND2-MetF and ND2-TrpR primers (Vella *et al.*, 2017). PCR conditions consisted of an initial denaturation of 3 minutes at 95° C, followed by 35 cycles of 30s at 95° C, 45s at 56° C and 45s at 72° C and a final extension step for 10 minutes at 72° C. All the amplicons were sequenced by the external provider Macrogen Europe (Amsterdam, Netherlands).

The new COI and NADH2 sequences obtained were imported and aligned with the CLUSTAL W algorithm (Thompson *et al.*, 1994) implemented in MEGA X (Kumar *et al.*, 2018). COI and NADH2 sequences of *R. alba* available from GenBank (NCBI) and BOLD databases (Ratnasingham *et al.*, 2007; Federhen, 2012; Sayers *et al.*, 2022) were retrieved to compare newly produced and already available sequences (SM\_1). Sequences were collapsed into haplotypes using DNASP v.6 (Rozas *et al.*, 2017). Lastly, the relationships among haplotypes and the reconstruction of haplotype networks were inferred with the TCS method (Clement *et al.*, 2002) implemented in the software PopART v.1.7 (Leigh & Bryant, 2015).

## Results

The eggcase was found on September 2nd, 2021 on a 42 m depth sea bottom in central western Sardinian coast (39.3580° N, 8.3048° E), while the white skate specimen was caught at a depth of 156 m in the northern part of the island (41.1592° N, 8.7817° E) on August 3rd, 2023 (Fig. 1A, B). The specimen was a female 132.1 cm long (TL) and weighed 16174 g (TM). Table 1 shows all morphometric measurements of the body are shown along with the teeth row count. Teeth appeared sharp and pointed cusped with a rather flat base (Fig. 1D). The specimen's jaws are deposited in the collection of the Zoological Mu-

seum of the University of Cagliari. The analysis of the reproductive organs revealed that the skate was immature in Stage 1. The stomach was found empty. The vertebral section analysis yielded good results in terms of growth band visibility, and the specimen was estimated to be 6 years old (Fig. 1E). Within the same haul, the white skate was caught alongside elasmobranch species. Specifically, the catch included 47 small-spotted catsharks [*Scyliorhinus canicula* (Linnaeus, 1758)], 14 thornback skates (*Raja clavata* Linnaeus, 1758) and 5 longnosed skates (*Dipturus oxyrinchus* (Linnaeus, 1758)).

The eggcase was found on a shallow, sandy sea bottom. The only other elasmobranch recorded in the same haul was a specimen of *Raja brachyura* Lafont, 1873. The eggcase (114.4 mm long, aprons excluded) (Table 1) appeared to be recently deposited as its surface did not present any sign of degradation or traces of epi-benthic colonisation. Moreover, once in the laboratory, it was dissected and found to contain undifferentiated yolk without a developed embryo.

DNA was successfully extracted, amplified, and sequenced for the eggcase and specimen retrieved in the Sardinian area, resulting in new COI and NADH2 sequences. Similarly, NADH2 sequences were obtained for four South African specimens, the same specimens analysed by Crobe *et al.* (2021). The sequences obtained during this study were deposited in GenBank (COI Accession Number: PP965249; NADH2 Accession Number: PP975989-PP978992) and used along with the public sequences found in online repositories (SM\_1).

The COI dataset (n = 36; length 630 base pairs) showed seven polymorphic and three parsimony sites, for a total of six haplotypes. At the same time, the NADH2 dataset (n = 11; length 910 base pairs) showed four polymorphic and two parsimony sites, for a total of four haplotypes.

The two samples, the specimen and the eggcase, share the same haplotype for COI (R\_alb\_H1), while two different NADH2 haplotypes characterise the specimen (R\_alb\_H2) and the eggcase (R\_alb\_H3).

In both COI and NADH2 datasets, *R. alba* haplotype networks showed a clear division between the northern hemisphere (i.e., Mediterranean Sea and Portugal) and the southern hemisphere (i.e., South Africa) (Fig. 1F).

## Discussion

The present study mainly aimed to confirm for the first time the occurrence of *R. alba* in the Sardinian waters by applying integrated taxonomy. Taking advantage of this significant finding, the capture of an immature specimen, we also report the collection on the second eggcase along the Sardinian Sea. In fact, Marongiu *et al.* (2021) previously reported the finding of a hatched eggcase of *R. alba*, suggesting the possible occurrence of the species outside its known distribution area. However, considering that such eggcase seemed to have hatched a long time before its discovery, together with the possibility that the currents could have transported this kind of structure after the hatching, the species' presence in the area could

**Table 1.** Morphometric measurements of *Rostroraja alba* specimen caught in Sardinian Sea expressed both as raw measurement (cm) and in percentage of total length (TL, on the left); On the right, *Rostroraja alba* new eggcase measurement in comparison with the eggcase described in Marongiu *et al.* (2021).

<i>Rostroraja alba</i> specimen			<i>Rostroraja alba</i> eggcase		
Morphometric measurements	Raw measurement (cm)	%TL		Raw measurement (mm)	
				Present study	From Marongiu <i>et al.</i> (2021)
Total length	132.1	-			
Disc width	97.5	73.81	Eggcase length	114.4	126.9
Disc length	76.2	57.68	Eggcase width	96.4	117.4
Head length (dorsal)	43.8	33.16	Anterior apron length	18.3	22.5
Head length (ventral)	42.9	32.48	Posterior apron length	15	11
Snout length (preorbital)	20.4	15.44	Left anterior horn length	109.5	130.1
Snout length (preoral)	22.8	17.26	Left keel width	11.3	14.5
Prenasal length	18.4	13.93	Left posterior horn length	60.9	77
Snout to maximum width	50.1	37.93	Right anterior horn length	116.1	132.5
Orbit diameter	3.9	2.95	Right keel width	11	14.5
Distance between orbits	10.6	8.02	Right posterior horn length	56	66
Orbit and spiracle length	7.6	5.75			
Spiracle length	3.2	2.42			
Distance between spiracles	10.3	7.80			
Mouth width	13.4	10.14			
Nare to mouth	4	3.03			
Distance between nostrils	12.65	9.58			
Width of first gill slit	2.4	1.82			
Width of third gill slit	2.8	2.12			
Width of fifth gill slit	1.9	1.44			
Distance between first gill slits	22.7	17.18			
Distance between fifth gill slits	15.5	11.73			
Length of anterior pelvic-fin lobe	13.6	10.30			
Length of posterior pelvic-fin lobe	25	18.93			
First dorsal-fin length	8.8	6.66			
First dorsal-fin anterior margin	6.1	4.62			
First dorsal-fin base	7.3	5.53			
First dorsal-fin height	3.9	2.95			
First dorsal-fin inner margin	1.7	1.29			
First dorsal-fin posterior margin	4.7	3.56			
Second dorsal-fin length	8.6	6.51			
Second dorsal-fin anterior margin	6	4.54			
Second dorsal-fin base	7.8	5.90			
Second dorsal-fin height	3.3	2.50			
Second dorsal-fin inner margin	1.2	0.91			
Second dorsal-fin posterior margin	4.5	3.41			
Tail width at axil of pelvic fins	6.7	5.07			
Tail width at tips of pelvic fins	6.2	4.69			
Width of tail across its midpoint	4.1	3.10			
Distance—snout to cloaca	71.3	53.97			
Distance—cloaca to 1st dorsal fin	41.9	31.72			
Distance—cloaca to 2nd dorsal fin	50.5	38.23			
Distance—cloaca to caudal-fin tip	60.8	46.03			
Number of tooth rows (upper jaw)	43				
Number of tooth rows (lower jaw)	45				

only be hypothesised at that time. Nonetheless, thanks to the data presented in this work, we are finally able to demonstrate the presence and the expansion in the distribution area of *R. alba*. Furthermore, the new eggcase was located fairly close to the one reported by Marongiu *et al.* (2021), indicating that the area may be a preferential deposition spot for the species (eggcase nursery). This could also imply that *R. alba* could also exhibit site fidelity behaviour, as suggested by Sousa *et al.* (2019) and observed for other batoids (Catalano *et al.*, 2021; Lavender *et al.*, 2022). Nonetheless, given the species' intrinsic rarity and the resulting lack of knowledge, future research using non-destructive approaches (e.g., ROV surveys and/or acoustic telemetry) should be conducted to clarify this aspect. Regarding the morphology, the new eggcase here described seemed quite similar in its proportions to the one from Marongiu *et al.* (2021), albeit slightly smaller in all dimensions. These discrepancies could be ascribed to the very different conservation stages of the samples, with one being long-hatched and the other recently deposited. However, the possibility that this difference may be due to a difference in size between the two egg-laying females cannot be discounted.

The white skate specimen was not caught in the same area where the eggcases were found but in the northern part of Sardinia Island on a deeper sea bottom (156 m), yet within the species' known depth range (from 40 to 500 m; Serena, 2005). The immature female showed a body size (132.1 cm TL) close to the size at first maturity (129.4 cm TL) indicated for the species by Kadri *et al.* (2014) in Tunisian waters. However, the specimen caught in Sardinia appeared much younger than what would be predicted for an individual of its size according to the von Bertalanffy growth curve provided by Kadri *et al.* (2014), which estimated ages ranging from 15-20 years for females with TLs of roughly 130 cm. Such discrepancy in growth could be ascribed to numerous factors. In fact, different environmental conditions, whether natural (e.g., prey availability, sea temperature) or human-driven (e.g., pollution or different fishing pressures), have been proven to affect fish growth rate (e.g., Aranha *et al.*, 2009; Wetherbee *et al.*, 2012). Nonetheless, besides the environmental conditions' effect, the possibility that the cause of this discrepancy is due to different methods or ageing criteria applied could not be ruled out (Carbonara *et al.*, 2019).

The DNA mitochondrial data highlighted that the two Sardinian samples (i.e., the eggcase and the immature individual) do not share the same haplotypes and hence, due to the matrilinear transmission of the mtDNA, do not share the same "mother". Therefore, it is reasonable to infer the presence of multiple adult breeders in the Sardinian seas. These findings can support the proposed extension of the species distribution area up to the northern Sardinian coasts. Skates are not new to the description of new and/or satellite nursery areas, such as seen in the recent findings regarding the genus *Bathyraja* in the Ross Sea (Finucci *et al.*, 2024).

The haplotype networks, despite the low number of sequences available, suggested that the Mediterranean pop-

ulation can be considered as a distinct population, with individuals genetically different from the South Africans. At the same, the presence of private haplotypes found in some areas could represent a further hint of a more complex genetic population structure to unravel at global and regional scales. Population separation has already been documented within and outside the Mediterranean Sea for sharks (i.e., Gubili *et al.*, 2016; Nikolic *et al.*, 2023) and skates (i.e., Catalano *et al.*, 2021), for which it has been suggested that peculiar life-history traits such as low fecundity and low dispersal potential (Griffiths *et al.*, 2011; Kousteni *et al.*, 2015) or geographical barrier could represent a limitation to the species connectivity (Catariño *et al.*, 2015; Di Crescenzo *et al.*, 2022).

The discovery of the critically endangered white skate in Northern Sardinia, an area that has already been identified as particularly important for other threatened elasmobranchs (Melis *et al.*, 2023a,b; Scacco *et al.*, 2023), emphasises the importance of increasing knowledge about the environmental dynamics that ensure favourable conditions for these species in the region, and thus providing all of the information required for their conservation. In fact, concentrating management and conservation measures in specific and strategic areas could produce multiple advantages (Bonanomi *et al.*, 2023). Additionally, the presence of juveniles, newborns (Scacco *et al.*, 2023), and eggcases (Marongiu *et al.*, 2021) of many species in the area may suggest the existence of attractive conditions for breeders and higher survival chances for young specimens, such as already hypothesised for other skates (Amsler *et al.*, 2015; Maguire *et al.*, 2023; Finucci *et al.*, 2024).

The Mediterranean Sea is considered one of the most important biodiversity hotspots (Coll *et al.*, 2010), particularly for threatened species such as elasmobranchs. For this, identifying and regulating important sites of value could counter declining population trends due to bycatch (Bradai *et al.*, 2022). Similarly, implementing measures in relatively confined areas could be more effective for the proper conservation of sharks and rays and eventually make these measures less impactful on fishing activities (Bonanomi *et al.*, 2023).

The application of new and remote monitoring technologies, such as ROVs, BRUVs and eDNA (Albano *et al.*, 2021; Leurs *et al.*, 2023), will surely provide essential information about the presence, abundance and habitat use of *R. alba* and other threatened species inhabiting the discussed areas. Furthermore, future investigations are needed to highlight the seasonal dynamics of these species in order to deploy effective marine conservation (Dunn *et al.*, 2023; Leurs *et al.*, 2023).

Despite the present paper being based on the record of a single specimen and a single eggcase of *R. alba*, Northern Sardinian coasts might still represent a site that should be protected and managed in the near future, especially considering the recent record of specimens of rare and endangered species in the same area (Marongiu *et al.*, 2021; Melis *et al.*, 2023a; Scacco *et al.*, 2023). In this context, future investigations and conservation-driven actions could be addressed under the umbrella of the 30

by 30 campaign (Kunming-Montreal Global Biodiversity Framework Target 3, 2023) and the Sustainable Development Goal 14: Life Below Water promoted by the United Nations (United Nations, 2015).

**Authors' contribution:** Andrea Bellodi and Simone Di Crescenzo contributed equally to this work and should be considered co-first authors.

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### Supplementary Material

The following supplementary material is available for this article:

**Table S1.** Data of already available and new COI and NADH2 sequences produced of *Rostroraja alba*. Each line represents a specimen.