# Multi-regional comparison of scarring and pigmentation patterns in Cuvier's beaked whales

Frazer G. Coomber[1,2] · Erin A. Falcone[3] · Erin L. Keene[3] · Gustavo Cárdenas-Hinojosa[4,5] ·
Rodrigo Huerta-Patiño[5,6] · Massimiliano Rosso[7]

## Abstract

Recent research on Cuvier's beaked whales (*Ziphius cavirostris*) from the Mediterranean has demonstrated that sexes can be visibly distinguished in photos using sex-linked patterns of scarring density and pigmentation, even at age classes which are notoriously difficult to differentiate. Being able to apply this research to other populations would allow for better monitoring of population demographics and vital rates globally. This study uses Photo Identification Captures (PICs) of known sex, adult Cuvier's beaked whales from three regions (Southern California, USA; Guadalupe Island, Mexico; and the Mediterranean Sea, Italy) to evaluate geographic variation in sex-linked patterns of scarring density and pigmentation. Standardized scarring density measurements from typical photo-ID views and Generalized Linear Models (GLM) were used to identify scarring density thresholds for sex at each region and for all regions combined to predict the sex of individuals. Scarring densities did not differ significantly among regions and thresholds calculated from any region correctly predicted the sex in other regions 92–98% of the time. An agglomerative cluster analysis with complete linkage identified three distinct pigmentation clusters in each of the three regions, with one being indicative of sex. This study supports the notion that scarring density is indicative of sex for this species, improves the predictive capacity of this metric inter-regionally, and provides a reliable method to estimate the sex of whales in a typical photo-ID catalog, thus supporting vital rate assessments for this data-deficient species.

**Keywords** Cuvier's beaked whale · Photo identification · Vital rates · Geographic comparison · Pigmentation patterns

## Introduction

The ability to accurately identify the sex and age of individuals within a population is an important aspect of cetacean ecology. The sex and age of a cetacean has been shown to influence social interactions (Weiss et al. 2021), the rate of inter-specific competition (Lee et al. 2019), and individual survival (Civil et al. 2018) and mortality rates (Ijsseldijk et al. 2020). Vital rates, relative changes in the survival and mortality parameters of a population over a set time period, can provide valuable insights into population health, and

✉ Erin A. Falcone
efalcone@marecotel.org

1   Mammal Society, Black Horse Cottage, 33 Milton Abbas, Blandford Forum, Dorset DT11 0BL, UK

2   The University of Sussex, Sussex House, Falmer, Brighton BN1 9RH, UK

3   Marine Ecology and Telemetry Research, Seabeck, WA, USA

4   Comisión Nacional de Áreas Naturales Protegidas, Ensenada, Baja California, Mexico

5   Proyecto de Investigación Zífido de Cuvier y Otros Cetáceos de Isla Guadalupe, Ensenada, Baja California, Mexico

6   Centro de Investigación Científica y de Educación Superior de Ensenada, Ensenada, Baja California, Mexico

7   CIMA Research Foundation, Via Magliotto 2, 17100 Savona, Italy

may reveal sub-lethal impacts of anthropogenic stressors before they result in a detectable population decline. They can be particularly useful when the onset or severity of effects may vary by age, sex, or reproductive status, and might be masked if these classes cannot be considered separately with available data (Booth et al. 2020). It has become commonly accepted that the use of mid-frequency active sonar (MFAS) poses a particularly high risk to deep diving species, especially beaked whales (Cox et al. 2006; Hooker et al. 2009). Vital rates are critical elements of energetic and Population Consequences of Disturbance (PCoD) models being developed for beaked whale species to estimate the long-term impacts of MFAS exposure on individuals and the populations they comprise (New et al. 2013; Pirotta et al. 2018). But despite this, many basic life history traits for these species remain poorly documented. Parameters including female calving interval, weaning age, age at sexual maturity, and reproductive lifespan are virtually unknown (MacLeod and D'Amico 2006; Baird 2019). These estimates of population parameters are typically identified through the use of long-term individual-based photographic identification studies (Clutton-Brock and Sheldon 2010; Karczmarski et al. 2022). However, the age and sex of individuals needs to be accurately determined and the sample representative of the population (Perrin and Reilly 1984).

There are multiple ways that cetaceans in the wild can be sexed, including behavioral associations (such as between a female and a calf, e.g., Rosso et al. 2011), direct observation of the genital region (e.g., Knowlton et al. 1994), and more recently through the genetic analysis of tissue biopsies (e.g., Shaw et al. 2003). Moreover, some cetacean species are dimorphic, displaying sexual differences in size (Caspar and Begall 2022), color, head/melon shape and the presence or absence of specific ornaments, e.g., erupted teeth (Mesnick and Ralls 2018). Therefore, many species can also be classified to sex using these distinctive differences. The Cuvier's beaked whale (*Ziphius cavirostris*) is one such species where adult males at the onset of sexual maturity develop erupted teeth (Heyning 1989), a trait that can be used to reliably sex an adult male at sea. However, Cuvier's beaked whales are notoriously difficult to study because they predominantly occur far from shore in small groups and can be difficult to detect during the very limited time they spend at the surface (Tyack et al. 2006; Barlow et al. 2006; Schorr et al. 2014). This makes sexing free-ranging Cuvier's beaked whales even more difficult, whether from biopsies (limited sampling opportunities), identification of erupted teeth (an individual's head may not be visible above the surface), or behavioral observations (only a subset of adult females are associated with a calf at any given time). Consequently, it is likely that many photographic catalogs of this species lack the essential classification of sex and age for a proportion of individuals. On the other hand, if photographed whales can be accurately classified to age and sex by appearance alone, these photographic data can form the basis of vital rates studies.

Sex specific differences in body scarring level and pigmentation patterns have been reported for a number of cetacean species (e.g., Martin and Da Silva 2006; Orbach et al. 2015; Lee et al. 2019) and have previously been used to determine the sex of wild animals from photographs (e.g., Rowe and Dawson 2009; Brown et al. 2016; Coomber et al. 2016a). Adult Cuvier's beaked whale males are quoted as being more heavily scarred than females (Heyning 1989) and a study conducted in the Mediterranean Sea was the first to quantifiably assess this statement using Generalized Linear Models (Coomber et al. 2016a). In doing so, it presented scarring density thresholds, values relating to the measured density of scarred to unscarred pixels from a photographic identification image, that could be used to reliably differentiate adult male (above threshold) and adult female (below threshold) animals (Coomber et al. 2016a). Inferences relating to sex-linked pigmentation patterns were also analyzed. However, this study was conducted on a limited sample size that used only high-quality, full-body photographic recaptures from a genetically distinct population (Dalebout et al. 2005). The current study, therefore, aims to expand this work by exploring if these methods are applicable across different geographic populations and if there are any geographical variations in these external characteristics, and also to develop the methods that can be applied more broadly to typical photographic identification images that do not include full-body sequences. To achieve this, the pigmentation classification and scarring density methods used previously will be applied to whales from two distant populations of Cuvier's beaked whales in the Pacific Ocean, one from Southern California, USA and the other from Guadalupe Island, Mexico. Scarring density threshold modeling and pigmentation cluster classification techniques were applied to a sample of independently sexed adults from each of these three populations, including the whales from the original Mediterranean Sea study, to examine the extent to which these diagnostic traits vary geographically within this widely distributed species, and which can further be applied to sex whales throughout the species range.

## Methods

### Data collection

A dataset of Photographic Identification Captures (PICs, the photographic documentation of a unique individual on a given date) of Cuvier's beaked whales was sourced from three geographic regions: the vicinity of the Southern California Antisubmarine Warfare Range, a MFAS training

area approximately 140 km off the coast of Southern California, USA (CAL), the oceanic Guadalupe Island located 260 km west of Baja California in the Mexican Pacific Ocean (GUAD), and the Ligurian Sea in the North-West Mediterranean Sea (MED) (Rosso et al. 2011; Coomber et al. 2016a; Fig. 1). In each study, groups of whales were approached with the goal of photographing each individual as completely as possible from the rostrum to the tailstock, though the minimum requirement for a successful PIC was one high-quality perpendicular view of either side of the dorsal body surface including the dorsal fin (See Appendix, Fig. A1). The CAL population has been the subject of dedicated photo-identification (photo-ID) research since 2006 (Falcone et al. 2009; Curtis et al. 2020). The regional catalog available for this study included 473 PICs of 250 unique individuals through 2019. Individuals in the GUAD population were photographed opportunistically as early as 2006 (Cárdenas-Hinojosa et al. 2015), with dedicated photo-ID research beginning in 2016. The available catalog from GUAD through 2019 included 677 PICs of 82 individuals. Individuals in the MED population were opportunistically photographed beginning in 1998, with dedicated effort since 2004 (e.g., Rosso et al. 2011). The MED catalog through 2019 was composed of 248 individuals documented by 2395 PICs.

Given the relative proximity of the study areas, identified individuals from the CAL and GUAD regional catalog were cross-examined to establish if any animal was present in more than one catalog.

From each PIC, a representative series of photos of one side of the body were selected for age classification (via visible diagnostic traits), visible body division, and scarring density measurement generally following the methods described in Rosso et al. (2011) and Coomber et al. (2016a), which used the same MED dataset considered here. Individuals showing erupted teeth were assumed to be adult males (hereafter AM) and toothless whales with paling on the head that surfaced in close association with a calf (a uniformly dark individual less than two-thirds the adult length) were categorized as adult females (hereafter AF), with sexes confirmed genetically when possible. Predominantly dark-bodied animals with little or no pale pigmentation on or near the head and that lacked erupted teeth and were not sighted in association with a calf were presumed to be immature and excluded from the study, along with apparently mature individuals that had no photographic documentation of these same sex-confirming traits.

The subset of analyzed PICs were collected between 2004 and 2019 and included 186 suitable quality PICs from 117
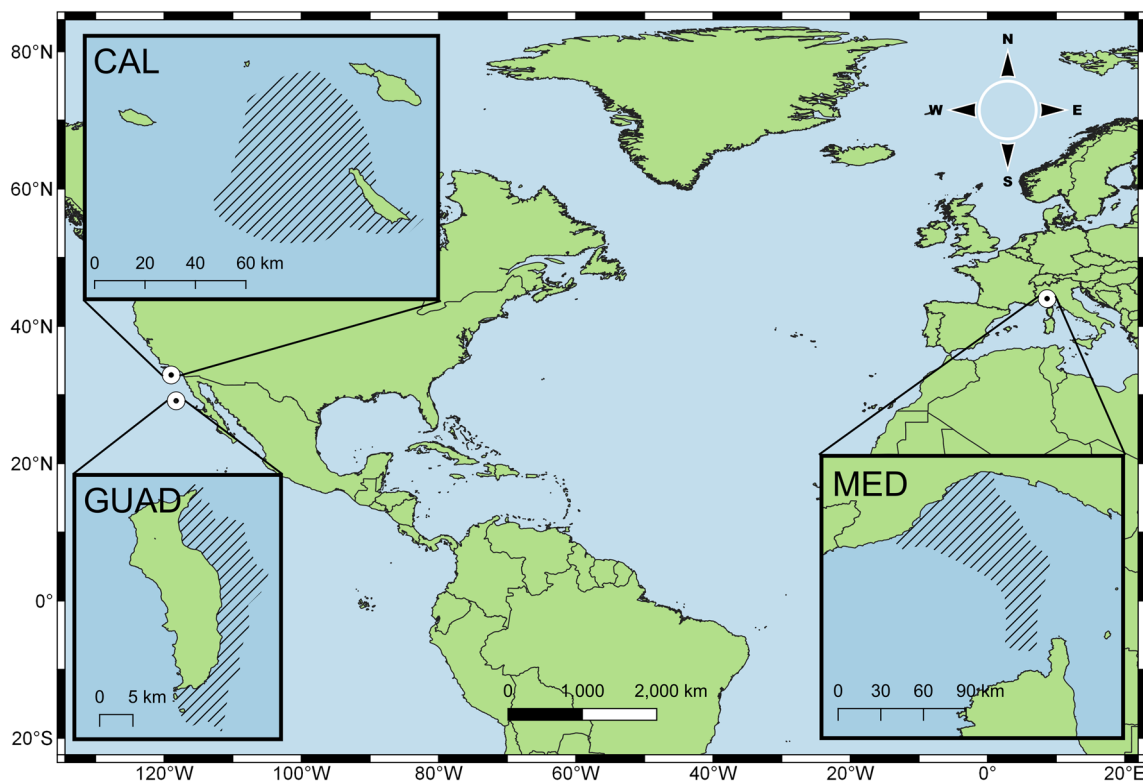


**Fig. 1** Global positions of the three geographic regions considered in the present study, with primary data collection areas for Cuvier's beaked whales within each region indicated by hashed areas in inset maps, including: Southern California, USA (CAL), Guadalupe Island, Mexico (GUAD), and the Mediterranean Sea (MED)

**Table 1** The total number of available photographic identification captures (PICS) of known-sex Cuvier's beaked whales by region with information on measured scarring densities

| Population | Number of PICs | Number of unique adult males (AM) | Number of unique adult females (AF) |
| --- | --- | --- | --- |
| CAL | 85 | 32 | 20 |
| GUAD | 53 | 11 | 6 |
| MED | 48 | 26 | 22 |

**Table 2** The final number of male and female individuals from each geographic region in the dataset used for the scarring density modeling, after the inclusion criteria were applied

|  | CAL ($n=36$) | GUAD ($n=13$) | MED ($n=48$) |
| --- | --- | --- | --- |
| Adult male (AM) | 26 (72%) | 7 (54%) | 26 (54%) |
| Adult female (AF) | 10 (28%) | 6 (46%) | 22 (46%) |

adult individuals of known sex (48 AF and 69 AM; Table 1) from all three regions. The set of representative photos from each PIC were independently quality scored (scale of 1–3 from best to worst), for the following three factors: proportion of the body visible above the water line, image exposure/lighting, and image sharpness/focus.

## Delineation of body into regions of interest (ROI)

The previous Mediterranean study (Coomber et al. 2016a) only used PICs that included images from the head to the tail stock in its analysis. These PICs were delineated into Regions of Interest (ROIs) based on the morphometric information that the length from the anterior to posterior insertion points of the dorsal fin is roughly one-seventh the distance from the blowhole to the anterior dorsal fin insertion in this species (Heyning 1989). Using ImageJ version 1.51g (Rasband 2011) and an image from each PIC that included both the blowhole and anterior insertion point of the dorsal fin, the visible body from the blowhole through the dorsal fin could be divided into seven equal sections ($ROI_{2-8}$). $ROI_{9-13}$ were delineated by extrapolating the width of these sections posteriorly. To expedite the delineation process for the two new catalogues (CAL and GUAD) and to allow the use of PICs that that did not contain an image that included both the blowhole and dorsal fin, a Bezier Curve Dividing Macro and an Angle Bisecting Macro (Supplementary Information 1; Eric Keen, Marine Ecology and Telemetry Research) were developed, and the distance from the anterior to posterior dorsal fin insertion points was used to divide the visible surface of the animal into standardized ROIs that were comparable to those in the MED data.

To ensure that data from the different geographic regions were comparable and to maximize the available sample size, the overall frequency distribution of available ROIs was calculated. This was achieved by counting the number of ROIs available from the 186 PICs of known-sex adult Cuvier's beaked whales. This frequency distribution indicated that PICs with data from $ROI_7$ to $ROI_{10}$ (the typical minimum photo coverage for a successful PIC; Fig. 2A) were most readily available, but limiting this selection to PICs containing either $ROI_{7-9}$ or $ROI_{8-10}$ increased the available sample size further (Fig. 2B). However, this dataset included

multiple PICs for the same individual. To ensure that there is no violation of independence within the sample, only one PIC from each individual was considered for subsequent analysis. In these cases, the PIC with the lowest combined quality scores, or the best overall PIC quality, was used. In the case of quality score ties from the same whale, the quality scores were prioritized with proportion visible as first priority and image exposure last. The $ROI_{7-9}$ was chosen for subsequent analysis as it provided the largest and most balanced dataset with 59 AM and 38 AF after the independence criterion was applied (Table 2). It should also be noted when interpreting results that this dataset is unbalanced with a bias toward AM.

## Scarring density threshold analysis

Intraspecific linear scars were identified and traced in ImageJ for each ROI of each individual using the photograph with the largest visible surface area for each available ROI, following the methods of Coomber et al. (2016a). The scarring density, the proportion of scarred to total pixels within a specific ROI or combined ROIs, was calculated by dividing the number of scarred pixels by the total visible pixels within a ROI.

All following analyses were conducted in R Studio version 4.0.3 (R Core Team 2020). To compare the observed mean scarring density between whales of different geographic regions, a one-way analysis of variance (ANOVA) was conducted. If the model assumptions of heterogeneity or normality of variance were violated, identified using a Levene's (Fox 2016) and Shapiro–Wilk's test (Royston 1982), respectively, the non-parametric Kruskal–Wallis equivalent (Hollander et al. 2013) was applied.

To investigate the predictive power of scarring density thresholds between geographic regions, models were fitted to the individual geographic regions and the resultant threshold used to predict the sex of known-sex animals from the other regions. All modeling was conducted within a binomial Generalized Linear Modeling (GLM) framework (Nelder and Wedderburn 1972) or its mixed effect extension (Pinheiro and Bates 2000), with the sex of the animal being the binary response (0 = female and 1 = male) and the scarring density (as the proportion of scarred pixels to unscarred pixels ranging from 0 to 1) within $ROI_{7-9}$ as the predictor
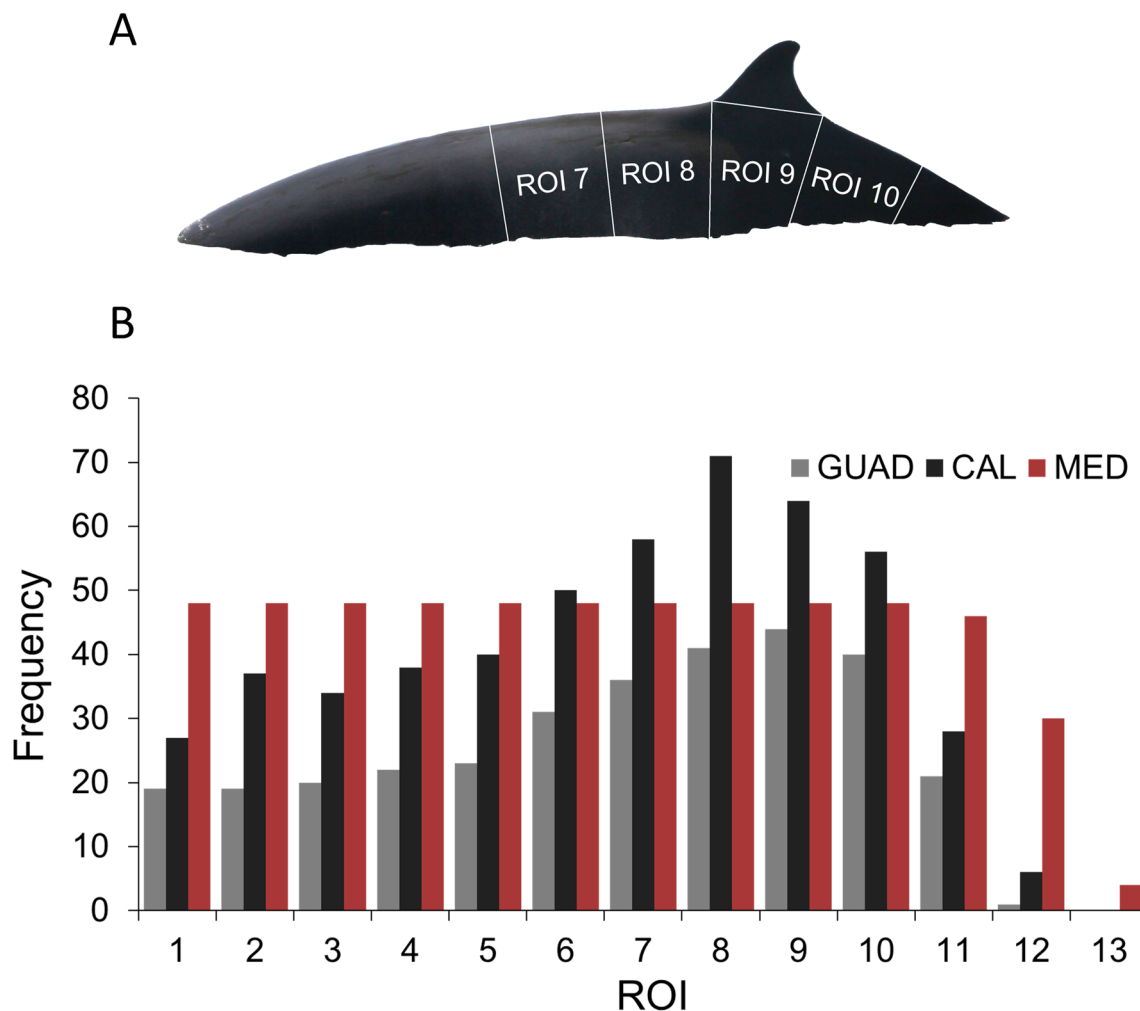
**Fig. 2 A** The typical body view of a Cuvier's beaked whale used for individual identification in most photographic studies, with the four most frequently measured ROIs in this study highlighted. **B** The frequency distribution of scarring density measurements by ROI for each study region, based on all available PICs. The MED study used only PICs that included the total body surface from $ROI_1$ through at least $ROI_{10}$. $ROI_7$–$ROI_{10}$ were the most frequently measured ROIs from CAL and GUAD

variable. The original model from Coomber et al. (2016a) was refit, due to recent genetic analysis indicating that one whale's sex was previously incorrectly identified (Baini et al. 2020). To determine the optimum scarring density threshold, Receiver Operating Curve (ROC) methods were used within the R pROC package version 1.17.0.1 (Robin et al. 2011). The threshold value, the scarring density at which individuals with a higher value should be classified as AM, was calculated based on the true positive rate (sensitivity) and true negative rate (specificity) of the MED training data. This threshold was then applied to the testing datasets from GUAD and CAL to predict the sex of adults based on their scarring density relative to the MED threshold at $ROI_{7–9}$. The discriminatory capability of the model was then measured using the Area Under the (Receiver Operating) Curve (AUC) statistic using the true known sex and threshold predicted sex of each individual. An AUC value of 0.5 indicates

that the model predicts no better than random and a value greater than 0.7 was deemed adequate for good model predictions. This process was then repeated using the data from CAL and GUAD separately as the model training data and the other geographic regions as testing datasets.

Additional candidate models with data from all geographic regions were fitted to further investigate if geographic region influenced the scarring densities and resultant thresholds. The models considered included: a full binomial GLM with no additional regional predictor variables, a binomial GLM with geographic region included as a fixed effect, and a binomial mixed effect GLM that included geographic region as a random effect. If geographic region was included in the best-fit model as a predictor variable, it would indicate that the scarring density threshold results are likely to differ by region and that a threshold from one region may not be applicable to another. To identify which of the candidate

models best fitted the full dataset, model evaluations were conducted using Akaike Information Criterion (AIC; Sakamoto et al. 1986), with the lowest AIC score being considered the best fitting model. Moreover, an ANOVA test was conducted between model residuals to see if the improved fit was statistically significant. The final model, based on the AIC and ANOVA results, was then fitted using combined data from all geographic regions to update the threshold analysis.

## Pigmentation data

Each PIC also provided information on external pigmentation features that were shown to be correlated with age and sex in the MED population. These generally followed the patterns defined in Coomber et al. (2016a) and included the presence and appearance of the following features: dark oval patches ("ovals") on the melon (Appendix Table A1), dark crescents ("crescents") posterior to the blowhole (Appendix Tables A2, A3), the color of the flanks (i.e., areas of the dorsal surface that were not depigmented, Appendix Table A4), and a depigmented cape extending posteriorly along the dorsal body surface (Tables A5, A6, A7, Fig. A2). However, a more complex set of cape descriptors was used here to better reflect the variability captured in the larger, multi-region sample of sexed adults (i.e., toothed males and reproductive females) in this study. In this case, where an extended cape was present, it was characterized by both its "pattern" (Table A5), i.e., the distribution of depigmented areas along the dorsal surface of the body, and its "texture" (Table A7), i.e., the appearance of those depigmented regions, in addition to its brightness (Table A6) and extent (last ROI to which the cape extends, Fig. A2).

A total of 217 PICs from 148 sexed adults included pigmentation data. However, not all PICs provided information on all pigmentation features; for example, PICs that did not include a good photograph of the head lacked oval data, and in some cases heavy scarring or diatom coverage precluded accurate characterization of the cape. Therefore, PICs were initially filtered to those that included data for the entire set of pigmentation descriptors. In the case where more than one PIC remained for an individual after this filtering, the photo quality criteria applied to the scarring density data

were also applied to the pigmentation dataset to yield a single representative data point for each whale. Following filtering, a total of 120 independent sets of complete pigmentation were available for analysis (Table 3).

## Pigmentation analysis

A hierarchical clustering method was applied to the filtered pigmentation dataset to accurately cluster animals with similar pigmentation patterns and differentiate these clusters from each other as much as possible. The first step was to calculate a dissimilarity distance matrix using the daisy function in R (Struyf et al. 1996) with the Gower metric (Gower 1971) in the Cluster package version 2.1.1 (Maechler et al. 2021). The advantage of the daisy function is that it computes dissimilarity matrices using mixed categorical, numeric, and binary data, among others (Struyf et al. 1996).

Two hierarchical clustering approaches were considered, a divisive and an agglomerative clustering method. Divisive represents a bottom-up approach, i.e., start with one big cluster that is subsequently divided based on dissimilarity. Agglomerative clustering—a top-down approach—starts with a number of clusters equal to the number of observations and subsequently groups clusters based on similarity until only one cluster remains (Ackermann et al. 2012). The complete linkage method was used as it finds similar clusters. This method uses the maximum calculated distances between clusters at subsequent stages from the dissimilarity matrix and merges clusters whose maximum distance is minimum (Sharma and Batra 2019). The agglomerative method with complete linkage tends to identify more numerous smaller clusters and was the method chosen for this analysis as it was hoped to provide additional insights to the pigmentation clusters defined in Coomber et al. (2016a). However, the divisive clustering approach, which typically identifies a smaller number of larger clusters, was also considered and the results are provided (Supplementary Information 2). Cluster analyses were conducted in R using the hclust function with a "complete" method for agglomerative and "diana" method for divisive clustering.

To identify the ideal number of clusters for both approaches, the silhouette coefficient was used (Rousseeuw 1986). This coefficient, which ranges from -1 to 1 with 1 indicating good consistency within clusters, was used to measure how close each point in a cluster is compared to the points in the neighboring clusters (Kaufman and Rousseeuw 1990). To test if the distribution of the resultant clusters was dependent on the sex or geographic region, a Chi-squared analysis was used.

**Table 3** The number of unique males and females from each region with complete pigmentation data that were included in the pigmentation cluster analysis

|                   | CAL ($n=35$) | GUAD ($n=15$) | MED ($n=70$) |
| ----------------- | ------------ | ------------- | ------------ |
| Adult male (AM)   | 23 (66%)     | 9 (60%)       | 41 (59%)     |
| Adult female (AF) | 12 (34%)     | 6 (40%)       | 29 (41%)     |

## Results

### Geographic region scarring density comparison

There were no individuals found in more than one catalog, allowing for a true comparison of scarring density between the independent populations from different geographical regions. Analysis indicated no significant regional differences in scarring density among AM ($\chi^2 = 4.83$, $df = 2$, $p = 0.09$) or AF ($\chi^2 = 2.80$, $df = 2$, $p = 0.25$) at the alpha level of 0.05. Even though the difference cannot be determined statistically due to a limited sample size ($n = 7$), GUAD AM exhibited higher average scarring density ($0.237 \pm$ SD 0.065) than those of AM in MED ($0.170 \pm$ SD 0.095) and CAL ($0.194 \pm$ SD 0.075). AF had very similar scarring densities, but those of CAL AF ($0.014 \pm$ SD 0.006) were slightly lower than those of MED ($0.026 \pm$ SD 0.022) and GUAD ($0.023 \pm$ SD 0.011; Fig. 3). Interestingly, females exhibited much smaller standard deviations when compared to males and there was no overlap between the distribution of scarring density by sex in both CAL and GUAD, whereas there was modest overlap in scarring densities between the sexes in the MED (Fig. 3).

### GLM of scarring density thresholds

The GLM fitted to the MED dataset indicated that any animal with a $ROI_{7-9}$ scarring density greater than 0.080 (equivalent to at least 8% of visible pixels within each $ROI_{7-9}$ being scarred) can be classified as an AM with reasonable confidence (~75% probability, Fig. 4A). Furthermore, this model indicated that animals with a scarring density greater than 0.250 are certainly AM, given the non-existent confidence intervals at this value. Using the 0.080 threshold value

derived from the MED model to classify the sex of independently known sexed CAL and GUAD animals resulted in near perfect predictions of all individuals (98%). The only exception was one CAL AM that was predicted as an AF. The AUC score of 0.985 indicates that the MED model predicts the sex of whales at other geographical regions much better than random.

Similarly, the model fit to the CAL data resulted in a lower scarring density threshold value of 0.043 (Fig. 4B), which correctly predicted the sex of 92% of the MED and GUAD testing animals with an AUC value of 0.913. All GUAD animals were predicted correctly by the CAL model, but one MED AM was classified as an AF and four MED AF as AM. The GUAD data fitted model, with a higher threshold value of 0.090 (Fig. 4C), predicted 92% of animals to the correct sex with an AUC value of 0.933. The animals that were incorrectly identified to sex by the GUAD model were all AM that were classified as AF.

Of the three candidate models considered for the combined scarring density data, the GLM with no regional fixed or random effects was shown to be the best fitting model based on the lowest AIC value (25.75), when compared to the GLMs with either fixed (28.96) or random effects (27.75) for region. Although the AIC scores were close, the difference was at least 2, indicating that adding a random or fixed effect did not improve model fit. Furthermore, the ANOVA Chi-squared test of the residuals indicated that the addition of a fixed effect did not significantly improve a model with no regional covariates ($df = 2$, $p = 0.67$).

Fitting the model to the combined dataset resulted in a scarring density threshold of 0.082 or 8.2% of pixels scarred that predicted the correct sex of nearly all animals (95%), incorrectly predicting five AM sexes as AF with an AUC score of 0.958 (Fig. 5). The inclusion of a larger dataset also

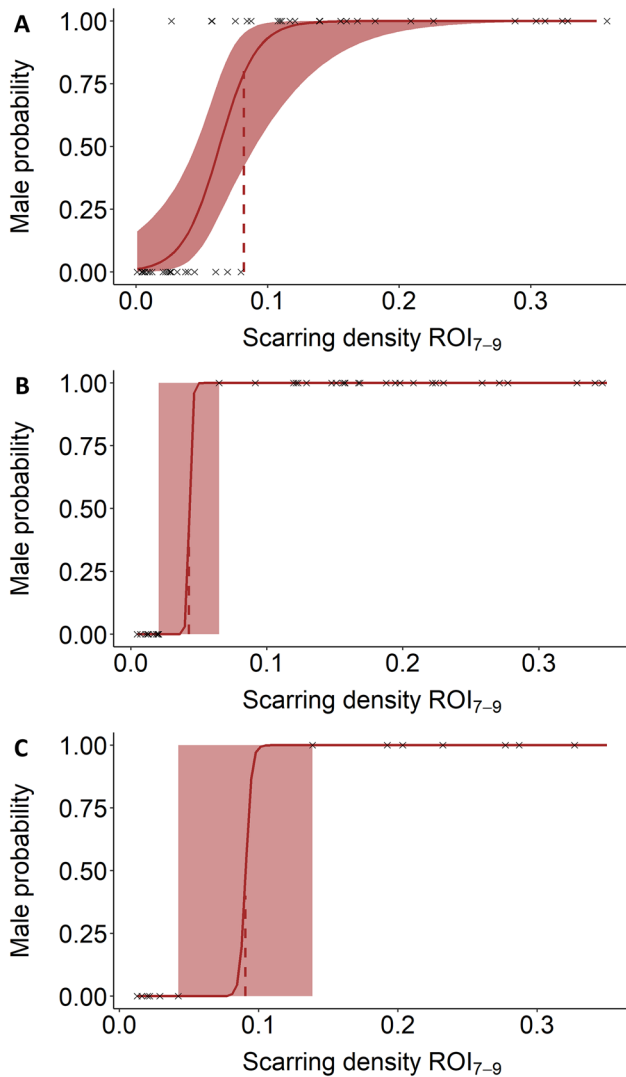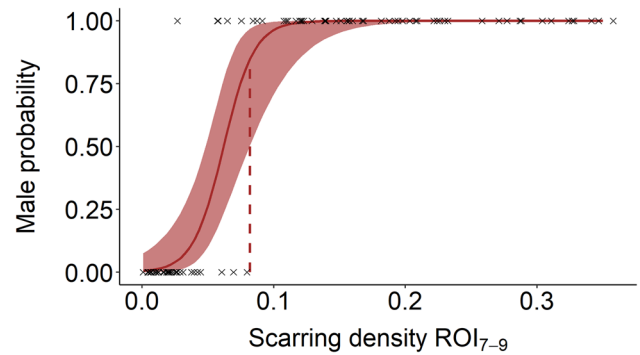**Fig. 3** Boxplots of the observed scarring densities for adult males and females by region

**Fig. 5** The best-fit model (binomial GLM with no fixed or random effects) of scarring density for males based on combined data from all regions, with the male scarring density threshold (0.082, dashed line) and the confidence intervals (shaded region) indicated. These thresholds are based on data from the dorsal fin zone (DFZ) of the body, in this case $ROI_{7-9}$. Cross marks represent the observed scarring densities for adult males (59 individuals, top) and adult females (38 individuals, bottom) in the filtered data

**Fig. 4** The GLM fitted to each of the three regional datasets, with the regional scarring density threshold cut-off for males from **A**—MED (0.080); **B**—CAL (0.043); and **C**—GUAD (0.090) indicated by dashed lines. Shaded regions represent the confidence intervals for the MED model and the region between the highest female and lowest male scarring densities for the CAL and GUAD models. These thresholds are based on data from the dorsal fin zone (DFZ) of the body, in this case $ROI_{7-9}$. Cross marks represent the observed scarring densities for adult males (top) and adult females (bottom) in the filtered data from each region (Table 2)

decreased the confidence intervals for the model (Fig. 5), such that any animal with a scarring density of at least 0.200 is certainly an AM, regardless of region.

## Pigmentation patterns

The agglomerative hierarchical clustering approach and the silhouette method, with a coefficient of 0.425, suggested that there were three distinct pigmentation clusters (Figs. 6, 7). The number of individuals and sexes for the three clusters



**Fig. 6** The results of agglomerative hierarchical clustering based on pigmentation features. Darker shades indicate a higher proportion of individuals in the cluster with a given appearance for each pigmentation feature considered
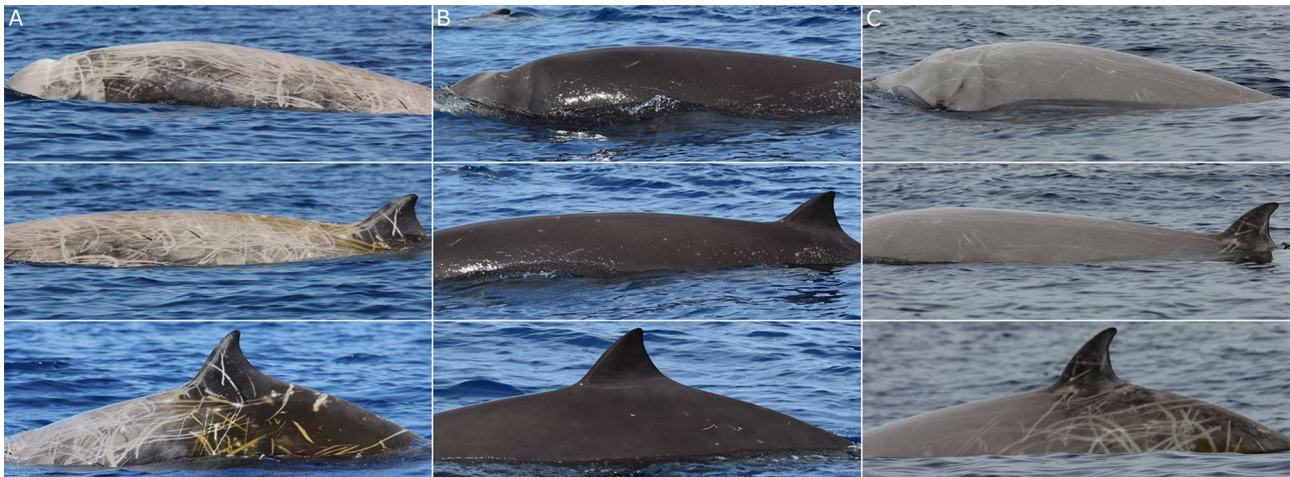
**Fig. 7** Examples of individuals in the three pigmentation clusters identified in the current study: **A** an adult male in Cluster 1, **B** an adult female in Cluster 2, and **C** an adult male in Cluster 3

**Table 4** The frequency distribution, including the percent of the animals within each cluster, by sex

|  | Adult male (AM) | Adult female (AF) |
|---|---|---|
| Cluster 1 ($n=35$) | 23 (66%) | 12 (34%) |
| Cluster 2 ($n=22$) | 0 (0%) | 22 (100%) |
| Cluster 3 ($n=65$) | 50 (79%) | 15 (21%) |

**Table 5** The frequency distribution, including the regional percent of the animals within each cluster, for each geographic region

|  | CAL ($n=35$) | GUAD ($n=15$) | MED ($n=70$) |
|---|---|---|---|
| Cluster 1 | 13 (37%) | 8 (53%) | 14 (20%) |
| Cluster 2 | 5 (14%) | 4 (27%) | 13 (19%) |
| Cluster 3 | 17 (49%) | 3 (20%) | 43 (61%) |

are summarized in Table 4. Cluster 1 included a high percentage of animals with ovals of varying appearance, distinctive long crescents, variable or discontinuous gray or white capes, and a medium or dark flank color. Cluster 2 had animals that had predominantly indistinct ovals and indistinct long crescents, a fading gray cape, and a medium flank color. Cluster 3 included animals that typically lacked ovals, had distinct long crescents, a white even cape of uniform texture and dark flank coloration.

The distribution of the sexes between the clusters was significantly different to an even distribution ($\chi^2=43.60$, $df=2$, $p<0.01$) with Clusters 1 and 3 having predominantly AM at 66% and 79%, respectively, and Cluster 2 being all AF (Table 4). All clusters were represented within each geographic region (Table 5), although the frequencies

were shown to differ significantly from an even distribution ($\chi^2=10.85$, $df=4$, $p=0.03$). However, the approximation of the test statistic may be subject to potential biases from the low expected values, especially for the GUAD animals. Furthermore, comparing the distribution of animals within clusters between just CAL and MED, with their larger sample sizes, the distribution of animals within clusters was not significantly different ($\chi^2=3.59$, $df=2$, $p=0.17$).

Within the available dataset, there were 47 animals (29 AM and 18 AF) that had scarring densities for $ROI_{7-9}$ and that were classified into a pigmentation cluster. When comparing the scarring densities of AM in Clusters 1 and 3 (Cluster 2 was not considered as it contained only AF), there was no significant difference ($F=0.28$, $df=1$, $p=0.60$). In fact, the average scarring density for Cluster 1 AM ($0.198\pm SD\ 0.072$) was very similar to that of Cluster 3 AM ($0.170\pm SD\ 0.093$), with both clusters having average scarring densities very close to the male certainty cut-off threshold of 0.2. In the case of AF, the scarring density was also not found to be significant among clusters ($F=2.49$, $df=1$, $p=0.13$), with average scarring density within Cluster 1 ($0.034\pm SD\ 0.032$), Cluster 2 ($0.024\pm SD\ 0.013$), and Cluster 3 ($0.014\pm SD\ 0.017$) being similar. However, great care should be taken when interpreting these results due to the low number of animals analyzed.

## Discussion

A previous study of Cuvier's beaked whales from the Mediterranean Sea found that scarring density thresholds from complete photographic sequences of the entire body surface can be used to reliably sex known adult animals from the same population (Coomber et al. 2016a). This study has

continued this work and confirmed that this method can also successfully be applied to at least two geographically distant Cuvier's beaked whale populations off the coast of California (USA) and at Guadalupe Island (Mexico). The findings that sex-based scarring densities do not differ significantly among these three geographic regions, that models fitted to distinct geographic regions can correctly predict the sex of nearly all known-sex adults from other regions, and that including geographic region as a covariate in a full model does not improve its fit all support this statement. Therefore, these findings suggest the plausibility of combining data across regions into a single model, which, when applied in this study, improved the model's predictive accuracy through the narrowing of confidence intervals.

The original Coomber et al. (2016a) study identified just two pigmentation clusters among known-sex adults in the Mediterranean sample: the pigmentation features in these "soft" and "sharp" clusters were comparable to Clusters 2 and 3, respectively, in the present work. The addition of more detailed pigmentation descriptors and additional individuals in the current study identified a third cluster. This new cluster, which is best described as whales with extended capes that are variable in color, pattern, and texture, included both males and females like the previously defined "sharp" pigmentation cluster. However, the uniquely adult female "soft" cluster (Coomber et al. 2016a) remained distinct in this larger, multi-regional sample of known-sex adults.

While all three clusters are present in all three regions, it remains true that only Cluster 2, the equivalent of the "soft" cluster, is diagnostic of sex in adults. This suggests that while pigmentation differentiation is a common trait in adults throughout the species range, only a limited number of adults (females in Cluster 2) can be reliably sexed by their pigmentation pattern alone. It should also be noted that a combination model, which included both pigmentation pattern and scarring density as predictors of sex, was considered for these data but failed to converge, due in part to the limited sample size of whales that had both complete pigmentation pattern classifications and scarring density metrics at $ROI_{7-9}$. Coomber et al. (2016a) successfully combined pigmentation and scarring density metrics in a common model based on full photographic sequences, but pigmentation characteristics were not retained during model selection, potentially due to the much higher predictive power of scarring density. Thus, while pigmentation differentiation remains necessary to confirm the onset of sexual maturity (Rosso et al. 2011), scarring density remains the more broadly applicable method of sexing adult whales (Coomber et al. 2016a).

In addition to developing several tools that greatly expedite the procedures required to consistently derive scarring density measurements (Supplementary Information 1), another important finding of this study was that complete photographic sequences were not required to correctly predict the sex of adult whales. We modeled sex-based scarring density thresholds using only the areas of the body just anterior to and below the dorsal fin. This is the typical view of the body that is used in photo-ID studies of this species and, thus, readily available for most whales in any study population. Given that it can be difficult to completely photograph individuals of this species in many circumstances, photos documenting tooth eruption and close calf association, and complete sequences from the rostrum to the tail are often lacking for many individuals in photo-ID catalogs. Indeed, the bias towards males in this study may be because all adult males have erupted teeth while only some adult females will have an attendant calf at any given time, limiting the opportunities to confirm their sex by this standard. It is also important to note that despite all efforts made to not violate the assumption of independence, there is a very small probability that a few animals within the dataset that do not have both a left- and right-side identification photograph may represent replicated samples.

Through the combined use of pigmentation differentiation cues associated with the onset of sexual maturity (Rosso et al. 2011) and the improved methods of deriving scarring density measurements presented here, many, if not most, Cuvier's beaked whales in a typical photo-ID catalog can now be efficiently sexed, with quantifiable confidence levels, using a single, good-quality identification image. The ability to reliably sex individuals in a population has important applications when looking at consequences of disturbance (Booth et al. 2020). Several studies have suggested that the effects of chronic disturbance on beaked whales are likely to be most evident in the female calving interval before they lead to reduced adult survival (Wright et al. 2007; New et al. 2013). While few, if any, ongoing Cuvier's beaked whale photo-ID studies can measure calving intervals directly for even a limited number of females (e.g., by documenting the same female in enough consecutive years to verify the births of all subsequent calves), this rate can be estimated through operational ratios of adult females to calves over shorter (e.g., annual) sampling intervals (Claridge 2013). Therefore, the ability to classify a greater number of adult females more confidently within catalogs, particularly those lacking other sexing characteristics (i.e., genetic confirmation or close association of a calf), will greatly improve operational ratio estimates.

It is worth noting that differentiating between adult females and sub-adult, or young adult, males is likely to remain challenging. These findings continue to suggest that female scarring densities are relatively low compared to mature adult males, especially in CAL and GUAD, and

males may exceed scarring density thresholds quite early in the maturation process; there are confirmed males in both the CAL and MED studies that exceeded them well before their teeth erupted, the cue used to confirm male sex in this sample (Rosso et al. 2011). In theory, rapid scar accumulation may inherently limit the number of younger males that can be confused with adult females in a population at a given time via this method, though further research is warranted. Although no gain rate analyses were conducted here, multiple sets of scarring density measurements exist for many of the whales included in this study, some over extended periods, and as has been done with other species (e.g., Mariani et al. 2016), these repeat measurements could form the basis of a scarring density gain rate analysis in the future. These gain rates could provide an additional data point to separate younger males from females, as scarring gain rates are higher in males than females of the same age for this species (Rosso et al. 2011). This difference in gain rate may explain why the standard deviation of scarring densities is much lower in adult females when compared to males as the variation in density between older and younger females is going to be less pronounced. An approach like this would be particularly relevant for known-sex adults that were also photographed as subadults.

Geographic variation in pigmentation patterns has been reported for other broadly distributed cetacean species, including spinner dolphins (*Stenella longirostris*; Perrin 1972), humpback whales (*Megaptera novaeangliae*; Rosenbaum et al. 1995), killer whales (*Orcinus orca*; Baird and Stacey 1988), and Indo-Pacific humpback dolphins (*Sousa chinensis*; Chen et al. 2018). In contrast, we found that while pigmentation patterns can be quite variable among individuals within a region, the same pigmentation clusters were present in all three distant populations of Cuvier's beaked whales. While the prevalence of these clusters may vary regionally, low sample sizes and expected values preclude accurate testing of the significance of these differences.

When generalizing these results to other populations, it must be remembered that comparisons were only made between three populations, and that only presumed intraspecific scarring was considered. The populations considered here differ dramatically in their spatial, ecological, and anthropogenic regimes. Despite falling within the boundaries of the Pelagos Sanctuary Marine Protected Area (Notarbartolo di Sciara et al. 2008), the range of the genetically distinct MED population (Dalebout et al. 2005) includes areas of high shipping traffic (Coomber et al. 2016b) and contains a number of individuals contaminated by persistent organic pollutants at levels associated with physiological effects in other marine mammals (Baini et al. 2020). Individuals in the CAL population inhabit an active military training area (Falcone et al. 2009; Curtis et al. 2020), where they are frequently exposed to MFAS and a range of other sound-generating activities known to disrupt foraging (Falcone et al. 2017). In contrast, the GUAD population inhabits a relatively undisturbed national Biosphere Reserve within Mexico (Cárdenas Hinojosa et al. 2015). Ocean-basin gene flow is low for this species (Dalebout et al. 2005), and while the CAL and GUAD populations are geographically much closer to each other than to the MED, there was no documented exchange of individuals between these two catalogs during this study. Given these ecological differences, the similarities in appearance among these three populations suggest it could be fair to assume that the scarring density model presented here is likely to reliably sex adults from other regions. However, it should be tested against a set of independently sexed individuals from any new study area before it is applied to an entire catalog. Further, incorporating other mark types, both naturally occurring (e.g., cookie-cutter shark scars) (Moore et al. 2003; McSweeney et al. 2007; Best and Photopoulo 2016) and anthropogenic in nature (e.g., entanglements and vessel collisions), into a future scarring analysis could provide additional insights into geographic variation in age-linked appearance traits for this species. For instance, anthropogenic scarring rates may reflect processes affecting population vital rates (Feyrer et al. 2021; Wickman et al. 2021).

In conclusion, this study has continued and improved upon previous work looking at the use of identification photographs of Cuvier's beaked whales to infer age and sex of individuals in long-term studies, a basic but necessary element for many ongoing conservation efforts. The main findings provide support that adult sex-specific patterns of pigmentation and scarring are highly similar between whales from very different geographic regions and as such can be used in combination to improve classification thresholds. These combined datasets, and the methods they have validated, offer the potential for more complex analyses in the future by increasing the quantity and resolution of age- and sex-specific life history data for this data-deficient species.

# Appendix: Cuvier's beaked whale pigmentation features

The following figures and tables provide detailed descriptions and examples of the pigmentation features applied to whales in the present study.

**Fig. A1** The dorsal flanks of two adult Cuvier's beaked whales off Southern California. This is the typical view used for individual identification in this species



## Ovals

See Table A1.

**Table A1** Ovals are patches of darker pigmentation on either side of a paler melon. They may vary in presence, contrast, and definition. Ovals can be difficult to accurately characterize without a high-quality photo of the melon. Ovals typically become less defined with age and can be lost completely or maintained well into adulthood

| Example | Category | Description |
|---|---|---|
| CAL-ID149  | None | Immature whales lack ovals because they have not begun the depigmentation process. Mature animals may lack ovals because the melon has become uniformly pale. |
| CAL-ID140  | Indistinct | Darker pigmentation gently fades into the lighter color of the melon. There is no clearly defined boundary and limited contrast between the pigmented and depigmented areas. |
| MED-ID30407282  | Distinct | High contrast and clear distinction between the light and dark areas of the melon. |

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## Crescents

See Table A2.
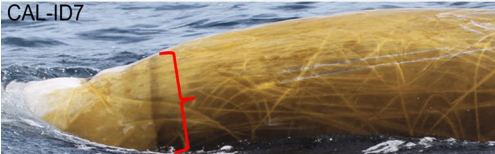
**Table A2** Crescents are bands of flank-color pigmentation posterior to the blowhole that are retained during the depigmentation process. They vary in contrast, length, number, and thickness among individuals, and within an individual as it matures. In general, crescents will start out indistinct and become more distinct with time; however, they may shorten over time and no longer be visible above the waterline in mature animals with a very pale cape

| Example | Category | Description |
|---|---|---|
| CAL-ID149  | None | Young animals that have not begun the depigmentation process will be uniformly dark and lack crescents. Crescents may also be secondarily reduced in older, very pale animals. |
| MED-ID31308233  | Indistinct | Low contrast crescents are common in younger animals with limited pigmentation differentiation. They may initially be indicated by regions of paling cape around them. |
| CAL-ID7  | Distinct | High contrast crescents are clearly visible against a pale cape. |

## Crescent length

See Table A3.

**Table A3** Crescent length describes how far the crescent extends from the flank to the top of the back. Crescent length changes as an animal ages and crescents may fade gradually along their visible extent. As a result, only the most distinct part of the longest crescent is scored

| Example | Category | Description |
|---|---|---|
| CAL-ID149 | None | Immature whales without crescents have no crescent length score. |
| CAL-ID77 | Short | Most distinct segment of crescent extends less than half the distance from the eye to the blowhole and may not be visible at all if whale is low in water. Can be "Distinct" or "Indistinct" and may fade to the top of the back. |
| CAL-ID92 | Medium | Crescents extend at least half the distance from the eye to the blowhole and are visible above waterline in typical surfacing. Can be "Distinct" or "Indistinct" and may fade to the top of the back. |
| CAL-ID7 | Long | Crescents extend to or near the topline of the back behind the blowhole and are clearly visible throughout their extent. |

## Flank color

See Table A4.

**Table A4** Flank color is the primary body color outside of the depigmented cape and scarring. In whales with extensive capes, it may only be visible in the crescents or ROIs posterior to the cape. It is difficult to accurately discern flank color in low-quality images or if a whale carries a heavy diatom load. Young animals tend to have medium flank color, and the flanks of most individuals either darken or lighten as they mature

| Example | Category | Description |
|---|---|---|
| MED-ID30412153 | Dark | Common in mature individuals and may be exhibited with any cape length. Individuals with this coloration have high contrast between the flank, crescents, and cape. |
| MED-ID30605031 | Medium | Common in immature individuals, but some adults retain this coloration even after cape differentiation. This coloration will sometimes contain a brown hue. |
| MED-ID30509201 | Light | Relatively uncommon; the darkest pigmentation on the body is pale gray, with limited contrast against the cape. |

## Cape pattern

See Table A5.

**Table A5** Cuvier's beaked whale capes range from non-existent to being so extensive the individual appears white. Individuals often express different cape patterns at different life stages, especially males. Cape pattern begins at the melon but excludes ovals and crescents. Without a complete view of the head and post-blowhole regions it can be difficult to accurately score cape pattern, and you may be unable to distinguish between "Discontinuous" and "Variable" patterns
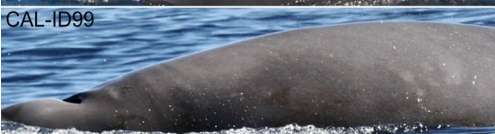
| Example | Category | Description |
| --- | --- | --- |
| MED-ID10505262 | None | Immature whales that have not started the depigmentation process have no cape pattern. |
| CAL-ID132 | Fading | Pale melon transitions to a cape that gradually but continually fades away along its extent, which is usually short. |
| CAL-ID122 | Discontinuous | Cape contains depigmented regions that are fully separated by flank color at one or more points along the back starting behind the blowhole and excluding the crescents. |
| CAL-ID77 | Variable | Cape is a continuous depigmented region along the body that varies in its shape and brightness throughout its extent and not just at the margins. |
| CAL-ID150 | Uniform | Cape is a single, continuous depigmented region that is consistent throughout its extent with the only variations occurring at the margins where the cape fades into the flank. |

## Cape color

See Table A6.

**Table A6** Cape color is the lightest color visible extending from the melon backward. Whales without a photographed melon cannot be scored. If present, cape color can range from gray to white. Diatom coverage and poor lighting can make cape color difficult to accurately score

| Example | Category | Description |
| --- | --- | --- |
| MED-ID10505262 | None | No cape present. Likely an immature individual and would not be able to be determined from a poor-quality image. |
| CAL-ID99 | Gray | Lightest depigmented areas are not white but grayish. This is common in younger animals, but a gray cape may be retained in adults. |
| MED-ID30309301 | White | Lightest depigmented areas of the body are white. |

## Cape texture

See Table A7.

**Table A7** Cape texture reflects the dominant appearance of the depigmented areas within the cape. Note that the margins of the cape vary in texture for most animals, this score reflects the body of the cape, especially along the top of the back that is visible in most ID images. Irregular cape textures are often associated with irregular cape patterns. If a cape appears mottled or wavy at the margins but is "Even" along the back, it should be scored as "Even"

| Example | Category | Description |
|---|---|---|
| MED-ID10505262 | None | Uniformly dark pigment along the entire dorsal surface of the body, no cape. |
| CAL-ID77 | Wavy | The cape includes irregular bands of pigmentation, typically in a "Discontinuous" or "Variable" pattern throughout its extent. |
| MED-ID30309301 | Mottled | Patchy, irregular depigmentation within cape, typically in a "Discontinuous" or "Variable" pattern. |
| CAL-ID150 | Even | Consistent depigmentation throughout the extent of the cape. All "Uniform" patterns are "Even" textured, as are some "Discontinuous" patterns. |

## Last ROI of cape

See Fig. A2.
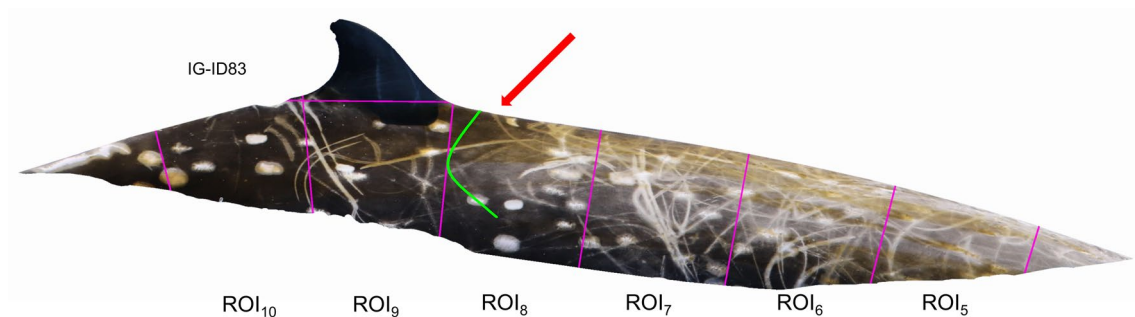


**Fig. A2** The last ROI of cape is furthest posterior ROI where depigmentation is present. Immature whales have no cape differentiation (last ROI of cape = 0), individuals just beginning to mature may have depigmentation only on the head (last ROI of cape = 1), and mature whales will have a cape extending beyond $ROI_1$, sometimes well past the dorsal fin. In this example, the cape extends to $ROI_8$, with its posterior margin indicated by a green line. Some capes fade softly into the flank making the end of the cape difficult to determine, especially in individuals with high scarring densities

## Declarations

**Conflict of interest** The authors have no competing interests to declare.

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