

# 3D bone analysis for improving species identification: a case study on scapulae of selected small toothed whales occurring along the Dutch coast

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**Abstract:** Carcasses are sometimes difficult to identify to species level when the head is lacking. The shoulder blade, or scapula, is often still well preserved in degraded carcasses though, and it can also be found in the archaeological and fossil record. Without any context, identification of these bones to the correct species can be cumbersome. In this pilot study, we investigated whether scapulae of small toothed whales (Odontoceti) possess sufficient unique features to discriminate between similar looking species. There is a clear need for this as illustrations of extinct and extant species occurring along the Dutch coast are available in outdated literature only. Photographs were taken from scapulae of 16 harbour porpoises (*Phocoena phocoena*), three common dolphins (*Delphinus delphis*) and three white-beaked dolphins (*Lagenorhynchus albirostris*). These were subsequently edited manually or semi-automatically. Overlapping pictures were aligned with specific software into a three-dimensional (3D) model. To compare the 3D models, 60 (semi-)landmarks were placed on each scapula. These landmarks were subsequently analysed with a newly created online bioinformatics pipeline ([github.com/naturalis/3d-cetacean-scapulae](https://github.com/naturalis/3d-cetacean-scapulae)). This pipeline produces a wireframe plot that visualises the differences between the bones investigated. The most discriminative components of the scapulae analysed could be identified. These components were the acromion, coracoid and glenoid cavity. An on-line interactive identification key was built ([cetaceanscapulae.linnaeus.naturalis.nl/](http://cetaceanscapulae.linnaeus.naturalis.nl/)) with these characters. With the underlying bioinformatics pipeline, many more bones of fossil and extant animal species found on the beach and in museum depots can now be analysed to improve their identification.

**Keywords:** automated image analysis, bioinformatics, cetaceans, interactive identification key, Linnaeus NG, photogrammetry, harbour porpoise (*Phocoena phocoena*), common dolphin (*Delphinus delphis*), white-beaked dolphin (*Lagenorhynchus albirostris*).

## Introduction

Carcasses are sometimes difficult to identify to species level when the head is lacking. The shoulder blade, or scapula, is often preserved in degraded carcasses though. It is also present in the archeological and fossil record and in bird pellets. The scapula has unique properties, enabling discrimination of different mammal species (Morgan 2009) and even sexes in humans (Papaioannou et al. 2012).

We investigated whether the scapula possesses useful characters to discriminate between similar looking species. As a case study, we chose species of small toothed whales (Odontoceti) that have been stranding on the Dutch coast for many centuries (e.g. Keijl et al. 2016, [www.walvisstrandingen.nl](http://www.walvisstrandingen.nl)). Reasons for this can vary from death by old age, illness, disorientation or marine debris (Unger et al. 2016). A carcass often strands in a degraded state or is incomplete due to fragmentation. This hampers the identification process. An incorrectly identified animal will influence stranding statistics (Keijl et al. 2016). The first step towards understanding why cetaceans strand is a correct identification. Isolated scapulae of small toothed whales also occur in the archaeological (van Deirse 1946, Bernal-Casasola et al. 2016) and fossil record (Heslinga & Schut 1971, Post & Kompanje 1995, Schouten 2017).

Identification of a single bone to species level can be done using various methods. A first method is comparing the bone with pictures in the literature or comparing it to museum specimens, preferably unmounted complete skeletons. This might not seem very time consuming for the relatively small number of species of small toothed whales recorded from the coastal regions of the Netherlands. Unfortunately, bones of these species are only depicted in rather obscure literature or literature using outdated taxonomic names (van Beneden & Gervais, 1868-1879, Benke 1993). Comparative material of rare taxa (e.g. *Mesoplodon*) is (virtually) absent or inaccessi-

ble in the natural history museums of the Netherlands and Belgium.

An alternative and very robust method is DNA-barcoding, by which DNA extracted from a bone is compared to that of known species (Ratnasingham & Hebert 2007). However, a molecular laboratory is required, and obtaining DNA-sequences of fossil material is cumbersome, expensive and may require dedicated (ancient DNA) facilities.

A third option is using an interactive identification key. This is a key where a user is aided by a software program to identify a specific organism with the help of images (Farr 2006). The user can choose a starting point for identification and can easily add or remove features for identification. With this method, it is possible to end up with the correct species identification even when only a single bone is used. Here we present a prototype of an online interactive identification key for whales and dolphins stranded along the Dutch coast to increase the accuracy and speed of identifications. We used Linnaeus NG, which is hosted by Naturalis Biodiversity Center, to build this key. The main advantage of Linnaeus NG is that it is web-based, so it runs on multiple platforms (including smartphones) and does not require the installation of additional software (Doorenweerd et al. 2014).

To analyse scapulae of Dutch small toothed whales, we used non-invasive three-dimensional (3D) analysis. 3D models are useful for other applications such as digitisation of collections and education. Various methods can be used to obtain 3D models of objects. The first one is CT-scanning. Compared to other methods discussed below, CT-scanning is expensive and requires moving the object to a machine elsewhere. Another way to obtain a 3D model is to scan an object with a 3D surface scanner. This method is more practical than a CT scanner, because a surface scanner is portable. The downside of surface scanners is that they perform poor on flat objects with sharp edges such as scapulae (data not shown). A promising third method that can

Table 1. Details of the scapulae that were used for this study. All scapulae were right-sided and derived from mature animals.

Species name	Museum	Sex	Specimen number
<i>Phocoena phocoena</i> Harbour porpoise	Natural History Museum Rotterdam	Female	NMR999000000159
			NMR999000000164
			NMR999000000304
			NMR999000000316
			NMR999000000324
			NMR999000000325
			NMR999000000335
		Male	NMR999000000341
			NMR999000000407
			NMR999000001425
			NMR999000000300
			NMR999000000303
			NMR999000000343
			NMR999000000403
<i>Delphinus delphis</i> Short-beaked common dolphin	Naturalis Biodiversity Center	Female	ZMA.MAM.2499
		Male	ZMA.MAM.11887
			ZMA.MAM.15.144
<i>Lagenorhynchus albirostris</i> White-beaked dolphin	Naturalis Biodiversity Center	Female	RMNH.MAM.35130
			RMNH.MAM.41152
			RMNH.MAM.41472

overcome the above-mentioned challenges is photogrammetry. With this method, a specimen is captured by taking overlapping photos, which are subsequently aligned on a computer to yield a 3D model. The method can be used to create accurate models of objects (Mallison & Wings 2014, Evin et al. 2016). As a first step, three similar looking cetacean species were analysed using photogrammetry to 1. generate 3D models; 2. carry out 3D analysis; and 3. identify components of the scapulae most suited to discriminate all three species.

## Materials & Methods

### Scapulae

For this project, scapulae deposited in the zoology collections of Naturalis Biodiversity

Center and the Natural History Museum Rotterdam were used. For commonly stranded harbour porpoise (*Phocoena phocoena*), scapulae of 16 adults (10 female, 6 male) were photographed. For the less common white-beaked dolphin (*Lagenorhynchus albirostris*), three scapulae (all female) were photographed. For the even rarer common dolphin (*Delphinus delphis*), three scapulae (one female, two male) were photographed. Sample size was limited but sufficient for finding discriminating characters for species delimitation other than size differences; investigating the influence of sex, function, evolution and ontogeny on scapulae traits as carried out by for instance Morgan (2009) was beyond the scope of our study. Details of the specimens analysed can be found in table 1.

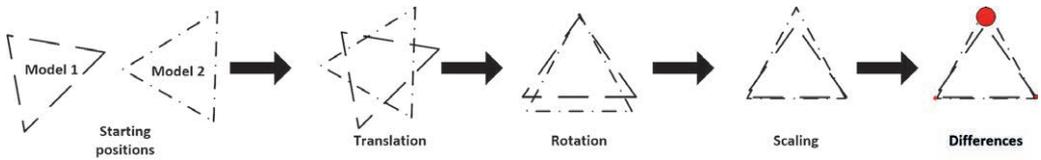


Figure 1. Procrustes analysis with two models. Each model has a different orientation. Procrustes translates, rotates and scales each model so that they obtain a similar form. This ensures that the forms can be compared (Gower 1975).

## Photogrammetry

Each scapula was photographed separately on a table with a sheet of black or green paper as background to facilitate masking and the removal of shadows. Several external sources of light were used to expose the scapulae, and to prevent additional shadows. A Nikon D300S camera was used mounted on a tripod to make high quality photographs. After taking a picture, a scapula was rotated slightly in the horizontal plane to create a full rotation. The scapulae were flipped after a rotation was complete to get full coverage of the front and rear sides (Mallison & Wings 2014). The images were edited manually or semi-automatically to apply masks. These masks allowed the photogrammetry software to distinguish background noise from the scapulae.

Manual editing was performed with Adobe Photoshop CS6 by selecting the full scapula with the Quick Selection Tool and by adding an alpha channel to each picture. This step was very labor intensive. Some scapulae had sufficient color difference from a black background to apply semi-automatic masking with ImageMagick convert (ImageMagick 1999). The conversion was used in a bash script that converted the black background of a picture to true black (RGB 0, 0, 0), which could be masked by the photogrammetry software.

Agisoft PhotoScan Professional was used to create 3D models from the masked images (Agisoft 2016). The software checked if there was overlap in input images and then aligned the images in a cloud model, which could be transformed into a 3D model with textures (figure 1). With the use of Python 3 (van Rossum 2012) it was possible to automate the dif-

Table 2. Position of the curves on the scapulae as shown in figure 3.

Curve number (Semi-)landmarks	Location
1	0 – 9
2	10 – 19
3	20 – 29
4	30 – 39
5	40 – 49
6	50 – 59

ferent steps of the process. An overview of the Agisoft workflow is shown in figure 2. Models that were created in the Agisoft software were exported as Polygon File Format (PLY) files. This file format can be used by landmarking software (Wiley 2007). More information about photogrammetry and Agisoft is available in de Vriend (2017).

## Landmarking

By using landmarking (Kendall 1984, Bookstein 1986), the bulging points resulting from the scans can be reduced from 100,000 points to less. Following standard procedures, a total of 60 empirically determined (semi-)landmarks were placed on the three models using Landmark (Wiley 2007). First of all, a total of three landmarks were placed on the base, apex and end of the inferior angle, axillary margin, glenoid surface, coracoid process, acromion, and superior margin each, that were assumed to be homologous because of similar anatomical origins in the three species investigated. Subsequently, seven nonhomologous semi-landmarks (Bookstein 1997) were added automatically connecting the three initial

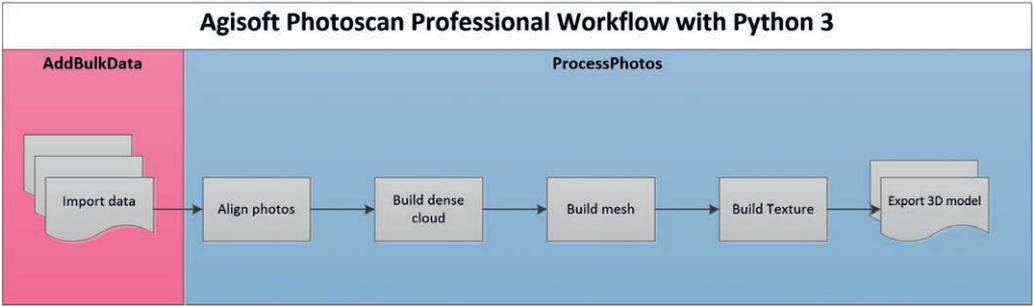


Figure 2. Schematic overview of the Agisoft workflow used in this project to create 3D models. Photographs in tiff format were imported with the AddBulkData script. The ProcessPhotos script created and exported a 3D model in PLY and PDF.

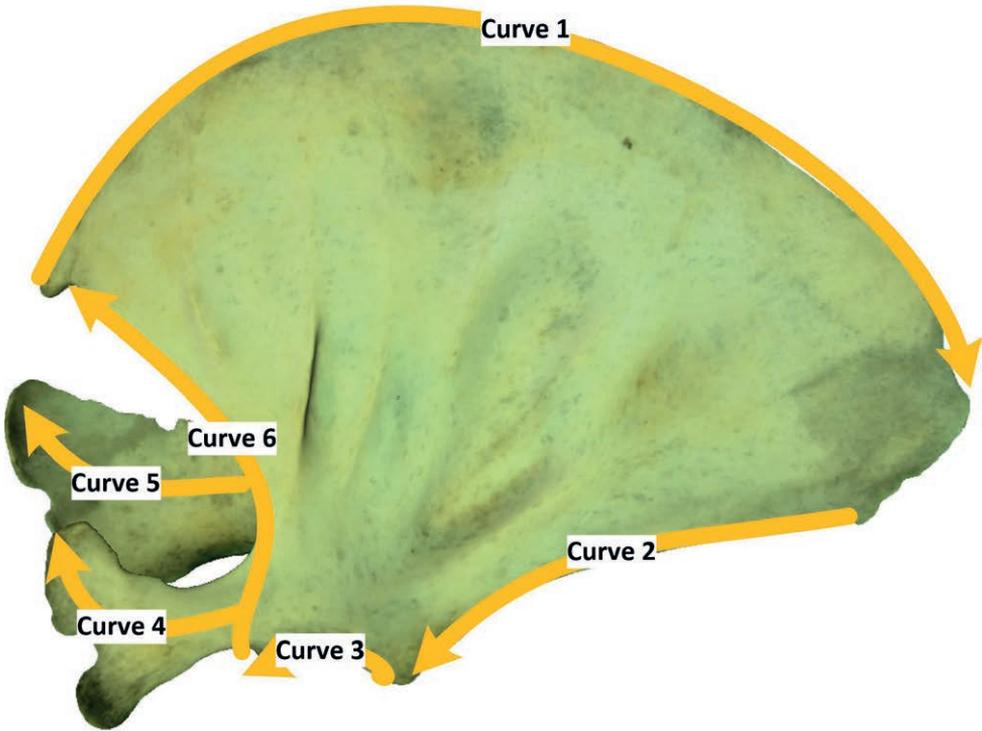


Figure 3. Curves that were placed on the scapula of a common dolphin (*Delphinus delphis*, ZMA.MAM.2499) using Landmark software. (Semi-)landmark numbers and location names can be found in table 2.

landmarks for the six separate regions on the scapula described above. The resulting curves are depicted in figure 3 and (semi-)landmark numbers and location names are shown in table 2. The (semi-)landmarks created were subsequently exported as a “dta” file.

**Bioinformatics pipeline**

The (semi-)landmarks that were placed upon the 3D models during this project were analysed with a newly created pipeline ([github.com/naturalis/3d-cetacean-scapulae](https://github.com/naturalis/3d-cetacean-scapulae)).

The pipeline was built in Galaxy (Afgan et

### 3D-Cetacean-scapulae galaxy

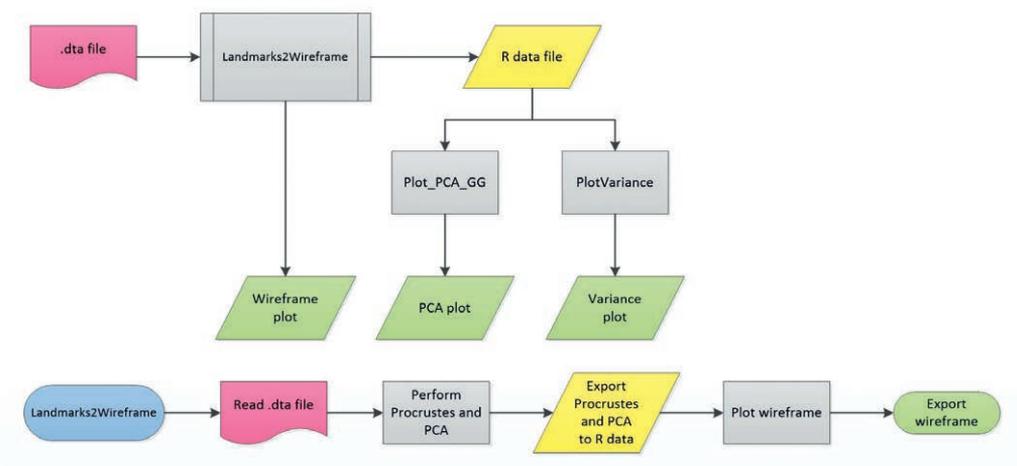


Figure 4. Schematic overview of the 3D-Cetacean-scapulae pipeline created. Upper part: flowchart of the entire pipeline. Lower part: flowchart of the Landmark2Wireframe module.



Figure 5. Scapulae of the three species currently included in the online interactive identification key. Images were made from 3D models in three different orientations. A: common dolphin (*Delphinus delphis*, ZMA.MAM.2499); B: white-beaked dolphin (*Lagenorhynchus albirostris*, RMNH.MAM.35153); C: harbour porpoise (*Phocoena phocoena*, NMR9990-0300).

al. 2016) and allows users to perform a 3D analysis on a dataset in an online platform. The pipeline first imports 'dta' files exported from Landmark Editor (Wiley 2007). A Procrustes and Principal Component Analysis (PCA) (Bookstein 1991) were subsequently performed using the R package Morpho (Schlager 2017). A Generalized Procrustes Analysis (GPA) was used to transform landmarks from 3D models to obtain the same orientation, and remove size so that only shape differences remain (Gower 1975). The aligned landmarks were processed with Procrustes and then used to perform a PCA. This method compares the variance in a dataset. Each dimension is called a principal component. The first component explains the largest variance, the second component explains the second largest variance and the last component shows the smallest variance. To show differences for each (semi-)landmark for each species, a wireframe plot was created. This was created on species level by calculating the mean position of each (semi-)landmark that was placed on all specimens for each species. The positions of the (semi-)landmarks in the wireframe plot were calculated using the loadings from the PCA. A mean shape for all specimens was obtained from the Procrustes analysis (Schlager 2017). Plots were created using the ggplot2 (Wickham 2009) package in R (R Core Team 2016). The entire workflow of the Galaxy pipeline is shown in figure 4. The most discriminative components in the wireframe plot were identified visually. These components were then converted into key characters for use in the Linnaeus NG key. Drawings explaining each key character for users of this key were made using Microsoft Office Visio 2010.

## Results

A total of 22 3D models of scapulae of harbour porpoise ( $n=16$ ), common dolphin (3) and white-beaked dolphin (3) were created.

For each species an example is shown in figure 5. According to the wireframe plots (figure 6), the three most discriminative components of the scapulae were the acromion, coracoid and glenoid cavity. With these, an interactive online identification key (cetaceanscapulae.linnaeus.naturalis.nl) was built using the character states described in figures 7, 8, 9 and 10.

Key to three species of Dutch small toothed whales based on characters of their scapulae

- 1 Base of acromion equally wide as apex (figure 8, right picture). Space between acromion and coracoid process larger than the width of either of these protrusions (figure 10, left side) — 2 1" Base of acromion narrower than apex (figure 8, left picture). Space between acromion and coracoid process smaller than the width of either of these protrusions (figure 10, right side) — *Delphinus delphis* (common dolphin)
- 2 Glenoid equally long as wide (figure 9, upper picture). Longitudinal ridges for muscle attachment on the dorsal and ventral surfaces of the scapula distinct (figure 5B) — *Lagenorhynchus albirostris* (white-beaked dolphin) 2" Glenoid longer as wide (figure 9, lower picture). Longitudinal ridges for muscle attachment on the dorsal and ventral surfaces of the scapula indistinct (figure 5C) — *Phocoena phocoena* (harbour porpoise)

## Discussion

Analysis of a total of 22 3D models enabled us to select the most discriminative traits of the scapulae for species discrimination. These models were all built with scapulae collected from adult animals as not enough juveniles and subadults could be sampled. It might be worthwhile to add data of immature animals as well because bones of juvenile or subadult animals are also found on the beach, in museum depots, and in the archaeologi-

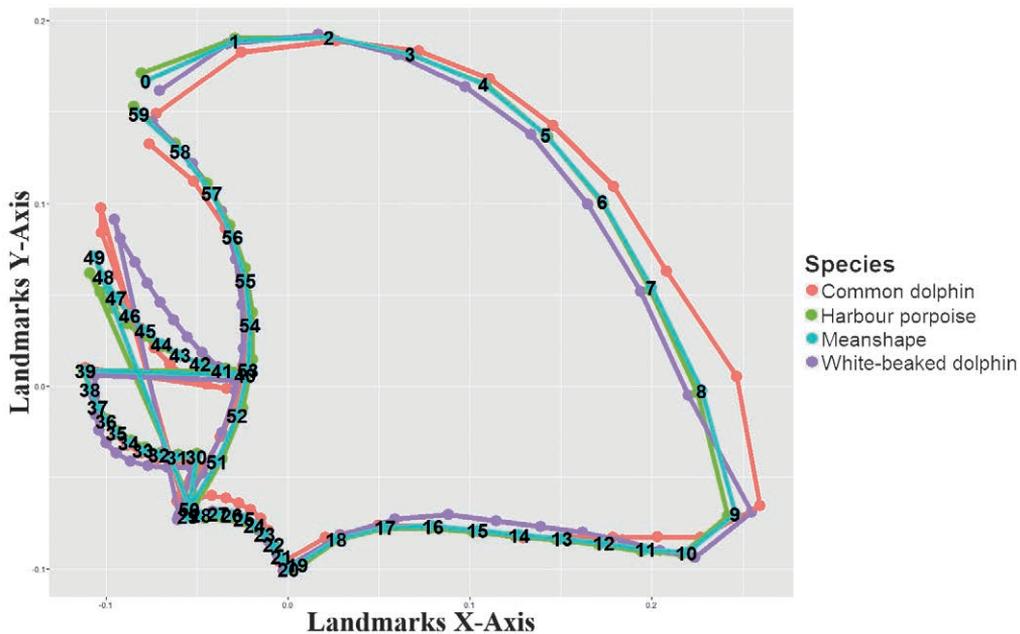


Figure 6. 2D wireframe of the mean groups for three scapulae of common dolphins (*Delphinus delphis*, red line), three scapulae of white-beaked dolphins (*Lagenorhynchus albirostris*, purple line) and 16 scapulae of harbour porpoises (*Phocoena phocoena*, green line) produced by the bioinformatics pipeline. The mean for all three species is indicated by the blue line. Each dot represents a (semi-)landmark.

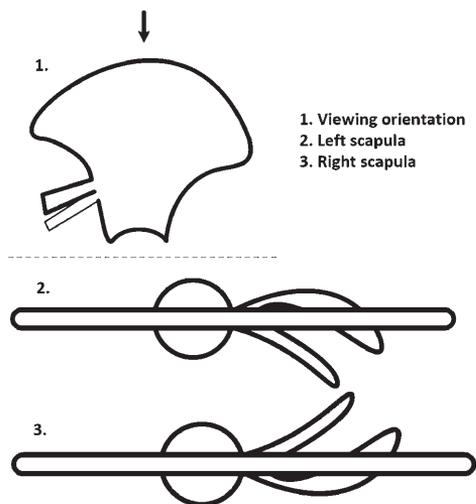


Figure 7. Explanation for identifying the left or right cetacean scapula. In distal view (1), the side can be identified by looking at the orientation of the acromion and coracoid process (2, 3).

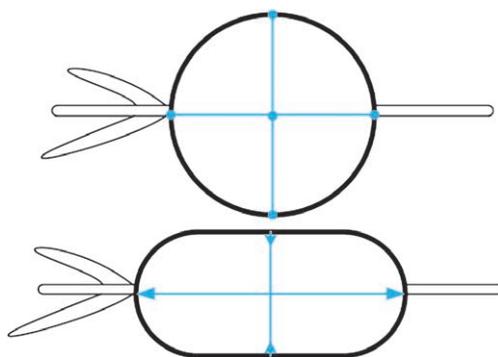


Figure 9. Illustration of the key character 'Shape of the glenoid surface in proximal view'. In the upper picture, the glenoid is equally long as wide. In the lower picture, the glenoid surface is longer than wide.

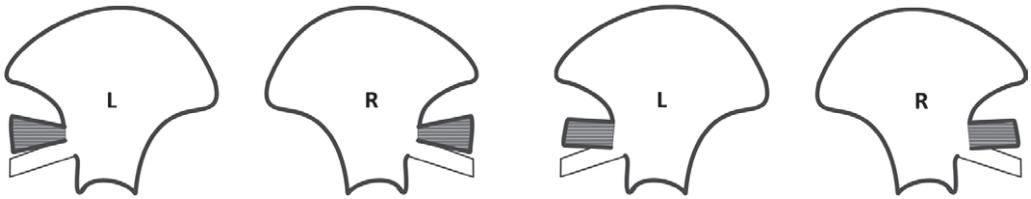


Figure 8. Illustration of the key character 'Shape of the acromion'. Left: base narrower than apex. Right: base equally wide as apex.

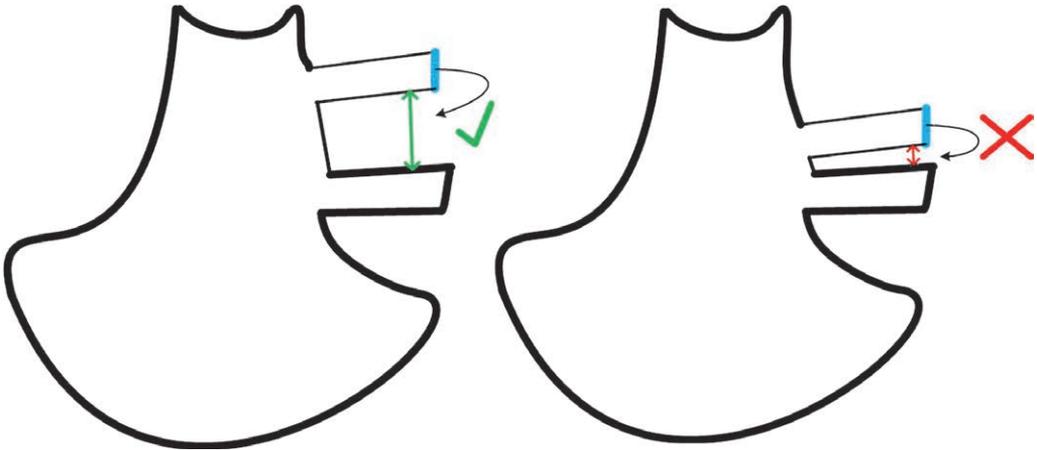


Figure 10. Illustration of the key character 'Space between acromion and coracoid process'. On the left, this distance, indicated in green, is larger than the width of either of these protrusions. On the right, this distance (indicated in red) is smaller than the width of either of these protrusions.

cal and fossil record. This could lead to misidentification when the interactive key is used on these animals. Data can be added by scanning scapulae of juvenile and subadult animals. New 3D models then have to be incorporated in the workflow described above to be used in interactive identification keys. We also restricted our analyses to species currently recorded from the Dutch coast, excluding the possibility of finding new species for this region, which might turn up due to the effect of on-going global warming. Data can easily be added by scanning scapulae of additional species though.

According to the wireframe depicted in figure 6, landmarks 0-9 in the inferior angle showed the largest differences among the scapulae of the species analysed. However, this part of the scapula is difficult to landmark and is likely to be fractured because scapu-

lae are very thin near the inferior angle. We therefore did not use this part for the diagnostic key. Landmarks 10-19 on the axillary margin did not give sufficient discriminative values due to too much overlap among the species analysed. Landmarks 50-59 on the superior margin did not yield informative results either for the same reason. Landmarks 57-59 showed some differences but are placed near the inferior angle so are prone to errors. Landmarks 20-29 placed on the glenoid surface showed some differences between species. The curve for the common dolphin showed no overlap with the other species near the end of the curve. Landmarks 30-39 placed on the coracoid showed a few unique differences for the white-beaked dolphin, and landmarks 40-49 on the acromion showed differences between the harbour porpoise and the two dolphin species.

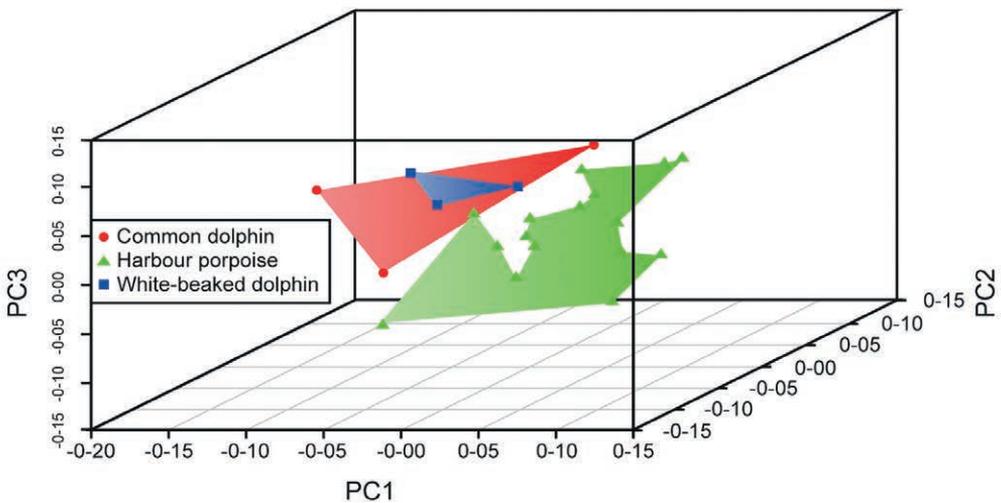
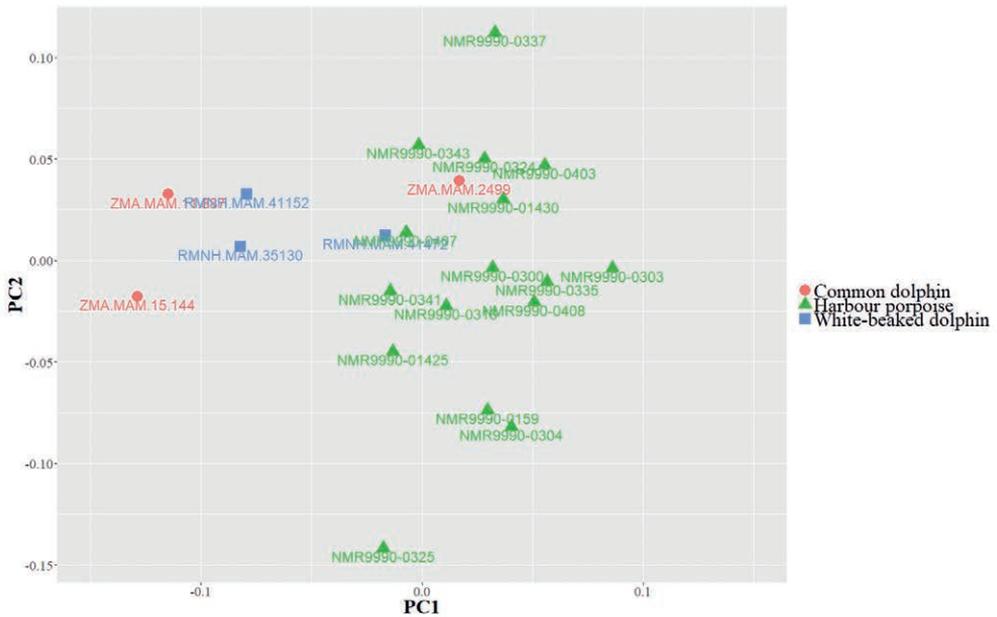


Figure 11. Results of a Principal Component Analysis of the 60 (semi-)landmarks of scapulae of 16 harbour porpoises (*Phocoena phocoena*, green triangles), three common dolphins (*Delphinus delphis*, red circles) and three white-beaked dolphins (*Lagenorhynchus albirostris*, blue squares). The x-axis shows the first principal component, the y-axis shows the second principal component, the z-axis shows the third principal component. Upper picture: Results of two-dimensional analysis. Lower picture: Results of three-dimensional analysis.

The results of a Principal Component Analysis in two dimensions showed that the individual scapulae analysed of the harbour porpoise ( $n=16$ ) cluster together. The scapulae of

the common dolphin ( $n=3$ ) and white-beaked dolphin ( $n=3$ ) have overlap with the harbour porpoise (figure 11, upper picture) but this overlap decreases in three-dimensional anal-

Table 3. Overview of cetacean species that stranded on the Dutch coast in the past two centuries.

Dutch name	English name	Scientific name	Family
Noordkaper	North Atlantic right whale	<i>Eubalaena glacialis</i>	Balaenidae
Blauwe vinvis	Blue whale	<i>Balaenoptera musculus</i>	Balaenopteridae
Noordse vinvis	Sei whale	<i>Balaenoptera borealis</i>	Balaenopteridae
Dwergvinvis	Common minke whale	<i>Balaenoptera acutorostrata</i>	Balaenopteridae
Bultrug	Humpback whale	<i>Megaptera novaeangliae</i>	Balaenopteridae
Gewone vinvis	Fin whale	<i>Balaenoptera physalus</i>	Balaenopteridae
Zwarte zwaardwalvis	False killer whale	<i>Pseudorca crassidens</i>	Delphinidae
Orka	Killer whale	<i>Orcinus orca</i>	Delphinidae
Gewone dolfijn	Short-beaked common dolphin	<i>Delphinus delphis</i>	Delphinidae
Griend	Long-finned pilot whale	<i>Globicephala melas</i>	Delphinidae
Witflankdolfijn	Atlantic white-sided dolphin	<i>Lagenorhynchus acutus</i>	Delphinidae
Witsnuitdolfijn	White-beaked dolphin	<i>Lagenorhynchus albirostris</i>	Delphinidae
Snaveldolfijn	Rough-toothed dolphin	<i>Steno bredanensis</i>	Delphinidae
Tuimelaar	Common bottlenose dolphin	<i>Tursiops truncatus</i>	Delphinidae
Gestreepte dolfijn	Striped dolphin	<i>Stenella coeruleoalba</i>	Delphinidae
Grijze dolfijn	Risso's dolphin	<i>Grampus griseus</i>	Delphinidae
Grijze walvis *	Gray whale	<i>Eschrichtius robustus</i>	Eschrichtiidae
Dwergpotvis	Pygmy sperm whale	<i>Kogia breviceps</i>	Kogiidae
Beloega	Beluga	<i>Delphinapterus leucas</i>	Monodontidae
Narwal	Narwhal	<i>Monodon monoceros</i>	Monodontidae
Bruinvis	Harbour porpoise	<i>Phocoena phocoena</i>	Phocoenidae
Potvis	Sperm whale	<i>Physeter macrocephalus</i>	Physeteridae
Spitssnuitdolfijn van De Blainville	Blainville's beaked whale	<i>Mesoplodon densirostris</i>	Ziphiidae
Spitssnuitdolfijn van Gray	Gray's beaked whale	<i>Mesoplodon grayi</i>	Ziphiidae
Gewone spitssnuitdolfijn	Sowerby's beaked whale	<i>Mesoplodon bidens</i>	Ziphiidae
Butskop	Northern bottlenose whale	<i>Hyperoodon ampullatus</i>	Ziphiidae
Dolfijn van Cuvier	Cuvier's beaked whale	<i>Ziphius cavirostris</i>	Ziphiidae

(\*) The gray whale went extinct in the Atlantic basin in historical times.

yses (figure 11, lower picture) currently not yet implemented in the bioinformatics pipeline. We expect that scapulae of the white-beaked dolphin and common dolphin might not overlap anymore when more scapulae of these two species are added to especially three-dimensional analyses.

The interactive identification key constructed in this study, and maintained by Naturalis Biodiversity Center, was built for just three species of Dutch cetaceans, so currently covers only 10% of all species stranding on the Dutch coast. More species now need to be added to the key, for an overview of all the stranded extinct and extant species see table 3. A good start would

be adding 3D models and traits of the Atlantic white-sided dolphin (*Lagenorhynchus acutus*), which is closely related to the white-beaked dolphin analysed in this study. Volunteers and/or students, affiliated with Naturalis Biodiversity Center or another institute with the appropriate permits, can easily add photographs of the remainder of the species so that the key can be expanded to cover the full spectrum of Dutch dolphin and whale diversity eventually. This study only used (semi-)landmarks placed on curves. Future studies could incorporate relative measurements as additional key characters when species are added with significantly larger-sized scapulae.

## Conclusions

By analysing 60 landmarks placed on 3D models of scapulae of three different species of Dutch small toothed whales, three regions on the glenoid surface, coracoid and acromion were identified as the most discriminative components. It will now be easy to expand the dataset with 3D models of scapulae of the remainder of the species of dolphins and whales stranding on the Dutch coast so that eventually all species can be keyed out. And with the same underlying bioinformatics pipeline, many more bones of other fossil and extant animal species found on the beach, in museum depots and bird pellets can now be analysed to improve their identification.

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## Samenvatting

### Hoe 3D-analyse de identificatie van botten kan vergemakkelijken: een demonstratie met schouderbladen van een selectie van Nederlandse kleine tandwalvissen

Karkassen zonder kop zijn soms lastig te identificeren. Het schouderblad, of scapula, in een karkas is echter vaak nog intact. Dit bot wordt ook los gevonden als fossiel op het strand, bij archeologische opgravingen, en in museumdepots. Zonder context is zo'n bot niet altijd makkelijk tot op de juiste soort te determineren. Wij hebben onderzocht of in het schouderblad voldoende onderscheidende kenmerken aanwezig zijn om sterk op elkaar lijkende soorten kleine tandwalvissen (Odontoceti) van elkaar te onderscheiden en op basis van die kenmerken een interactieve identificatiesleutel te maken. Daar is veel behoefte aan omdat illustraties van skeletten van dolfijnen en walvissen alleen te vinden zijn in verouderde literatuur. Er zijn 3D modellen van 22 scapulae gemaakt van museumobjecten van 16 bruinvissen, drie gewone dolfijnen en drie witsnuitdolfijnen met fotogrammetrie. Vervolgens is een bio-informatica pijplijn gebouwd ([github.com/naturalis/3d-cetacean-scapulae](https://github.com/naturalis/3d-cetacean-scapulae)) om de verschillen tussen de onderzochte soorten te visualiseren. Daarmee zijn onderdelen van scapulae geïdentificeerd die het meest geschikt zijn om de verschillende soorten walvissen en dolfijnen van elkaar te onderscheiden. Drie componenten van de scapulae bleken voldoende informatief voor het onderscheiden van de verschillende soorten, namelijk het schouderdak (acromion), het ravenbekuitsteeksel (processus coracoideus) en het gewrichtsvlak (glenoid). Deze onderdelen zijn gebruikt om een interactieve identificatiesleutel in Linnaeus NG te maken, die

op internet te vinden is (cetaceanscapulae.linnaeus.naturalis.nl/). Met dezelfde bio-informatica pijplijn kunnen nu ook andere botten geanalyseerd worden om identificatie tot op

de juiste soort te vergemakkelijken.

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