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## RESEARCH ARTICLE

# Whale Bone Puzzles: Reconstructing and Identifying Historical Whale Skeletons Using Archive Records, Osteology, and Zooarchaeology by Mass Spectrometry (ZooMS)

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Museum collections not only provide educational tools for the public, but also reference material for osteological research and baseline information for understanding historical population dynamics and food webs. Such applications are only possible, however, with accurate identifications of museum osteological specimens, which is sometimes challenging, as specimens can be separated from their original information. In order to clarify missing information about provenience and species identifications, we analysed 13 historical whale bone specimens from the Museum of Zoology, Strasbourg, using a multidisciplinary approach that combined historical document analysis, osteology, and Zooarchaeology by Mass Spectrometry (ZooMS). These analyses enabled identification of elements from seven different whale species: five baleen whales (Mysticeti) and two toothed whales (Odontoceti). Two skeletons could be connected with letters from the early 1900s indicating they derived from whales stranded on the shores of South Island, New Zealand. One of these skeletons was digitized using a 3D scanner and can be freely viewed online. All of the samples will be available through a digital archive. The identification and digitization of these museum whale specimens, which include threatened and endangered whale species whose past histories are not yet fully understood, is of great value and ensures they are fully available for future systematic research. The study demonstrates a new and useful application of ZooMS, particularly in concert with other methods, to support museum collections research.

**Keywords:** museum collections; historical whales; archives; bone; mass spectrometry; conservation biology

## 1. Introduction

The Museum of Zoology in Strasbourg (MZS) grew out of the 18<sup>th</sup> century natural history cabinet of Jean Hermann (1738–1800), a French physician and naturalist who was a professor of botany, philosophy, '*materia medica*' and

natural history. After his passing, the collections were purchased by the City of Strasbourg, marking the origin of the museum, and have since been significantly expanded. The museum was under French administration until 1871, when Alsace was annexed by Germany. A period of German administration followed, until 1918 when Alsace was retroceded to France in the aftermath of World War I. In 1893, under German administration, the collections were split between separate zoological, mineral, and botanical sections under the Kaiser-Wilhelm University in Strasbourg, and a building specifically devoted to the zoological collections was inaugurated. The zoological collections are still housed in this building today and have expanded to encompass some 1.2 million specimens, including more than 10,000 mammals. With the notable exception of insects, the specimens were mainly collected from across the world during the 19<sup>th</sup> century (review in Wandhammer 2008).

In the process of relocating the collection prior to the future renovation of the museum, we examined whale bones that had been preserved for decades on storage

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shelves and never properly inventoried. The investigation of these bones is part of a broader inventory of all the historical mammalian specimens in the museum so they can be entered into the Global Biodiversity Information Facility (GBIF) international database (Flemons et al. 2007; Telenius 2011). Initial investigation of the whale bones involved consultation of historical documents and osteological analysis. Morphological analyses did not allow for identification of all elements to a genus or species level.

Consideration was then given to the possibility of bio-molecular-based identification of the whale specimens, which can improve identifications. One possible approach is analysis of ancient DNA (aDNA). DNA-based study can easily distinguish between different cetacean species (Speller et al. 2016). However, this method is expensive and DNA is often contaminated or degraded during museum preparation procedures or over long periods of storage, meaning that trying to extract and study aDNA can be costly and/or difficult (Linderholm 2016; Leonardi et al. 2017; Pedersen 2016; Foote, Hofreiter, and Morin 2012). Recently, protein-based identification approaches have emerged as a rapid, high-throughput, and cost-effective alternative for taxonomic identification of archaeological material. Zooarchaeology by Mass Spectrometry (ZooMS) uses peptide mass fingerprinting of collagen found in bone, scale, hide and skin, or of keratin found in baleen and feathers, to taxonomically identify animals (Buckley 2018; Hendy et al. 2018; Buckley et al. 2009). The approach has been used to successfully identify archaeological cetacean bones (collagen) to genera and Mysticeti whale baleen (keratin) to species (Solazzo et al. 2017; Buckley et al. 2014; Evans et al. 2016; Speller et al. 2016; Hofman et al. 2018). Although primarily used to identify archaeological bones, ZooMS has also been successfully employed to explore historical archives made of parchment and vellum (Fiddyment et al. 2019, 2015). However, its use in aiding identifications in osteological museum specimens has been so far limited.

Using a combination of historical documents, traditional osteological analysis, and ZooMS to put together the jigsaw puzzle of whale bones at the MZS, we were able to reconstruct four largely complete skeletons and identify various additional skull and skeletal elements. In addition, we were able to confirm the origins of two of the whale skeletons. Altogether we identified elements from seven different whale species: five baleen whales (Mysticeti) and two toothed whales (Odontoceti).

## 2. Material and Methods

### 2.1. Assessment of collection

The entire collection at the MZS was screened to gather all cetacean material. These materials were further assessed and samples with known provenance and/or well-recorded information which did not require further investigation were not included in the study. From the samples with incomplete information, all of the bones from baleen and toothed whales were selected for further analysis. Three bone sets bore a pencil mark on each bone: I, II or B.

### 2.2. Historical documents

Several lines of historical documentary evidence were used to identify when the samples were collected. First, the labels on the bones and shelves were consulted along with anything that was with the bones, such as newspapers used as wrapping. Second, the available museum catalogues and registers were consulted to find any reference to whale entries or their identifications. Third, existing correspondence from curators and directors was consulted when considered likely to contain relevant information due to the date of entry.

### 2.3. Osteological analysis

The bones were individually removed from the shelves and a full osteological analysis was conducted. For each bone, where possible, the element, age class (adult/juvenile), approximate size, and taxonomy were determined using comparative anatomy according to published morphological characteristics (Van Beneden and Gervais 1880; Jefferson, Leatherwood, and Webber 1994) and comparative reference material from Muséum National d'Histoire Naturelle (MNHN), Paris. In addition, the condition of each bone was assessed, including colour and texture. For the skeletons, classical techniques were applied to identify the bones, lateralize paired elements such as ribs and long bones, and determine the position of vertebrae and ribs. The completeness of the skeletons was determined and any bones that did not appear to be part of a single individual were removed and considered separately.

### 2.4. Peptide mass fingerprinting (ZooMS)

After the study of available documents and osteological analysis, a subset of the unidentified bones was sent for analysis by ZooMS in order to improve the resolution of taxonomic identification. Subsamples of 14 bones from seven individuals weighing 10–30 mg were taken and placed in 1.5 ml Eppendorf tubes at the MZS. Subsequent processing was conducted at the Max Planck Institute for the Science of Human History, Jena. Samples were demineralized in 250–500  $\mu$ l of 0.6 M hydrochloric acid (HCl) at 4°C. Following centrifugation, the supernatant was removed, and the samples were rinsed three times in 200  $\mu$ l of 50 mM ammonium bicarbonate solution ( $\text{NH}_4\text{HCO}_3$ ) pH 8.0 (AmBic) and then incubated in 200  $\mu$ l of 0.1 M sodium hydroxide (NaOH) for five minutes at room temperature to remove compounds which interfere with mass spectrometry. All solutions were aqueous using ultrapure water. The samples were vortexed and centrifuged, and the supernatant discarded. The samples were again rinsed three times in AmBic. Finally, 100  $\mu$ l of AmBic were added to the samples, followed by incubation for one hour at 65°C to gelatinize the collagen. Samples were then centrifuged and 50  $\mu$ l of the supernatant was incubated overnight at 37°C with 0.4  $\mu$ g of trypsin. Following digestion, samples were acidified with 1  $\mu$ l 0.1% trifluoroacetic acid (TFA) and purified using a 100  $\mu$ l C18 resin ZipTip® pipette tip (EMD Millipore) with conditioning and eluting solutions composed of 50% acetonitrile and 0.1% TFA and a lower hydrophobicity wash buffer of 0.1% TFA. Collagen peptide fragments were eluted in 50  $\mu$ l (Brown et al. 2020; Buckley et al. 2009; Welker et al. 2015).

The collagen peptides were analysed via Matrix-assisted laser desorption/ionization time of flight mass spectrometer (MALDI-TOF-MS) in order to identify biomarkers. The collagen was diluted 1:10 with eluting solution and then 0.5  $\mu$ l of the diluted trypsin-digested extract was mixed with 0.5  $\mu$ l of  $\alpha$ -cyano-hydroxycinnamic acid and spotted in triplicate with calibration standards onto a 384-spot MALDI target plate and analysed on a Bruker autoflex speed MALDI-TOF-MS. Spectra were visually inspected using the mMass software (Strohalm et al. 2008) and all samples produced high-quality spectra with high signal-to-noise ratios and multiple discrete peaks (SI1 Figure 1). Replicate spectra from each of the samples were then identified to taxonomic groups using published markers (Buckley et al. 2014). Spectra were uploaded to Zenodo (doi: 10.5281/zenodo.3746000).

### 2.5. Overall identifications

Based upon the information gathered from all three sources, skeletons were reconstructed using the newly identified bones using the following criteria: pencil marks, age class, size, condition of bones, and species. Where possible, information was combined to achieve the best taxonomic classification for each bone or set of bones.

## 3. Results

### 3.1. Description of the bones

Fifty-seven sets of bones, baleen plates, and teeth belonging to cetacean species were recorded within the collection (SI2 Table 1). The majority of the sets were easily identified to species. This included four sets identified as baleen whales and 24 sets identified as toothed whales, many of which were Delphinidae. Thirteen baleen and toothed whales that were not clearly identified were chosen for further analysis in order to determine the species, gather historical and geographical data, and attempt to reconstruct skeletons. These 13 samples included one complete skeleton, three partial skeletons, one single bone, and eight skulls or skull fragments (**Table 1**). The remaining 16 sets were too fragmentary to be prioritized for species-level identification.

### 3.2. Documents and historical data

There were few labels or other information unambiguously attached to the 13 specimens chosen for further analysis. In addition, even if labels were included, it was difficult to trust their accuracy because the collections were moved multiple times over the past 150 years, sometimes without the labels.

Labels were clearly associated with four of the samples (**Table 1**). MZS Mam11114 is a bone fragment with an old label reading “*Balaena mysticetus*, zum Schädel gehörig” (*Balaena mysticetus*, belonging to the skull). MZS Mam11118 is a cranium that has been and still is, on display in the entrance to the museum, maybe since its opening in 1893 (**Figure 1a**). It has a rather recent label, from the 1980s according to the plastic it is made of, reading “*Balaena mysticetus*”, but we found no original information on this sample. MZS Mam11109 is a partial skeleton with a handwritten tag attached to

the atlas reading “Vertèbre cervicale de Rorqual du Cap *Balaenoptera*...” (cervical vertebra of a Cape whale). The last word is unreadable. MZS Mam11111 is another partial skeleton with all the bones marked with a ‘B’. Alongside this is an old wooden box, presumably the original shipping case, which contains several baleen plates and a label “Walbarten zum Skelet B, auf dem Boden, *Balaenoptera sulfurea*, Neu-Seeland” (baleen of skeleton B, on the floor, *Balaenoptera sulfurea*, New Zealand). In addition, two tympanic bullae wrapped in an old newspaper are also marked ‘B’. The newspaper is a 1905 issue of *New Chivalry*, a New Zealand journal. Taken together, these documents are in favour of MZS Mam11111 being from New Zealand circa 1900. One additional faded label which unfortunately was not associated with any sample was retrieved. It reads “Potwal ... Madagascar Voeltzkow”, indicating that sperm whale (Pottwal in German) parts were provided by Alfred Voeltzkow (1860–1947) at some point. Voeltzkow was a German zoologist who sold a number of specimens from Madagascar to the museum of Strasbourg in 1901. Finally, for three specimens, MZS Mam11094, MZS Mam11103 and MZS Mam11119, taxonomic information was found on paper inventory cards, which had been the standard inventory form of the German period (1871–1918), but here the handwriting is clearly more recent (after WWII) so this is unlikely to be original information.

Old catalogues provided three references to whale bones. Unfortunately, none can be directly linked to any particular skeleton. Two references come from the register. One entry in October 1883 reads, “201: Embryos von *Balaenoptera Sibbaldii* von Vardoe durch Dr Guldberg. Preis 410 M.” This indicates the entry of ‘embryos’ of a whale from Vardø in Finnmark county, Norway, through a Dr Guldberg, and the price is given in Reichsmark. ‘Embryos’ is a strange term in this context. We have encountered several such mentions in our historical catalogues when references to skeletons are made. We suggest that ‘Embryo’ here could refer to a skeleton in 19<sup>th</sup>-century German, but we have found no external confirmation of this. The second entry in 1906 reads “3 Walfischskelette (Dall, NeuZealand) 600 MK” referring to three whale skeletons in 1906 from a Mr Dall in New Zealand, for 600 Reichsmark (which roughly corresponds to current 3500 €). In addition to registers, there are two lines in a catalogue from the German period in the list of ‘Cetacea carnivora’ which read, “321 *Balaenoptera Sibbaldi* (*Balaena*, *Sibbaldius borealis*), Skelett, Vardoe (Norwegen). 321a. Rechte obere Extraemitaet. 321b. Zähne aus dem Oberkiefer. 321c Schaedel.” This indicates the presence of a whale (*Balaenoptera borealis* or sei whale according to *Mammal Species of the World*) skeleton: right forelimb, teeth from the upper jaw and skull. This is rather odd since a sei whale would have no teeth. A whole series of entries are missing in our archives: some 825 items from the first 20 years of German administration (1871–1893) are absent. Numbering starts with 826 in one entry register and we have not found the previous one, which may explain why the whales are so poorly documented.

A third set of documents containing references to whales was the correspondence of Ludwig Döderlein, prominent curator and director of the museum (1882–1919) during

**Table 1:** Combined identifications of the various whale bones at the MZS.

Invent #	Element(s)	Element for ZooMS	Historical ID	Osteological ID	ZooMS ID	Overall ID	Common Name
MZS Mam11094	mandible	n/a	<i>Physeter catodon</i>	<i>P. macrocephalus</i>	n/a	<i>P. macrocephalus</i>	sperm
MZS Mam11103	mandible-half	mandible	<i>Balaena mysticetus</i>	<i>B. mysticetus</i>	<i>E. glacialis/B. mysticetus</i>	<i>B. mysticetus</i>	bowhead
MZS Mam11104	upper cranium	cranium		Mysticeti (parvorder)	<i>B. physalus</i>	<i>B. physalus*</i>	fin
MZS Mam11105	mandible parts	n/a		<i>B. physalus</i>	n/a	<i>B. physalus*</i>	fin
MZS Mam11106	mandible	n/a		<i>P. macrocephalus</i>	n/a	<i>P. macrocephalus</i>	sperm
MZS Mam11109	vertebrae and ribs	vertebra	<i>Balaenoptera</i> sp.	Mysticeti (parvorder)	<i>B. physalus</i>	<i>B. physalus*</i>	fin
MSZ Mam11110	.1 complete skeleton	cranium		<i>M. novaeangliae</i>	<i>B. acutorostrata/B. bonaerensis</i>	<i>B. bonaerensis**</i>	minke
	.2	mandible			<i>B. acutorostrata/B. bonaerensis</i> or <i>B. borealis</i>		
MZS Mam11111	.1 skeleton	vertebra 16	<i>Balaenoptera sulfurea</i>	<i>B. musculus?</i>	<i>B. acutorostrata/B. bonaerensis</i>	<i>B. bonaerensis**</i>	minke
	.2 without skull	rib 5L			<i>B. acutorostrata/B. bonaerensis</i>		
	.3	left arm			<i>B. acutorostrata/B. bonaerensis</i>		
	.4	haemal arch			<i>B. acutorostrata/B. bonaerensis</i> or <i>B. borealis</i>		
	.5	sternum			<i>B. acutorostrata/B. bonaerensis</i>		
	.6	scapula			<i>B. acutorostrata/B. bonaerensis</i>		
MZS Mam11112	complete skeleton	n/a		<i>B. arnuxii</i>	n/a	<i>B. arnuxii</i>	Arnoux's beaked minke
MZS Mam11113	.1 partial skeleton	cranium		Mysticeti (parvorder)	<i>B. borealis</i>	<i>B. borealis</i>	minke
	.2	vertebra			<i>B. borealis</i>		
MZS Mam11114	bone fragment	n/a	<i>Balaena mysticetus</i>	<i>B. physalus</i>	n/a	<i>B. physalus*</i>	fin
MZS Mam11118	cranium	cranium	<i>Balaena mysticetus</i>	<i>E. glacialis</i>	<i>E. glacialis/B. mysticetus</i>	<i>E. glacialis</i>	right
MZS Mam11119	cranium and upper jaw	n/a	<i>Physeter catodon</i>	<i>P. macrocephalus</i>	n/a	<i>P. macrocephalus</i>	sperm

\* Indicates elements that possibly belong to the same individual.

\*\* Indicates elements that likely belong to the same individual.



**Figure 1: Old documents related to whale specimens at the MZS. (a)** Post-card, probably from the beginning of the 20<sup>th</sup> century (first published in 1923), showing the entrance hall of the museum. **(b)** Notes by Ludvig Döderlein at the back of a letter from James Dall dated Oct 8, 1905.

most of the German period. We found the letters which James Dall sent to Döderlein to organise the purchase and shipment of three whales, obviously those mentioned in the register, from New Zealand to Strasbourg via London. Dall owned a horticultural business in Nelson, New Zealand, and collected plants for sale, occasionally selling natural history specimens to European museums (S11). Döderlein notes at the back of one letter that the museum buys: “1. complete skeleton of *Berardius arnuxii*; 2. complete skeleton of *Sibbaldius sulphureus*; 3. incomplete skeleton of *Epidon novaezealandiae*. All three together to the price of £30.” (Figure 1b).

More information about these three skeletons comes from two further letters. A letter from Dall on October 8, 1905 states, “at the present time I have a skeleton of *Berardius Arnuxii* which is perfect with the exception of one tooth which someone had removed” that had “washed up on the Para Para Beach in Collingwood New Zealand – January 1905”. He speaks further about the species, in a letter dated June 21, 1906: “I send you a Postcard with a picture of a remarkable fish, some say it is a species of

Dolphin. One gentleman calls it *Berardius Arnuxii* others say something else. I understand it comes out of Pelorus or Queen Charlotte’s Sounds, & meets nearly every steamer on the Nelson to Wellington run & has been there now for a number of years – of course it is difficult to get a good photo with the fish underwater; it probably comes to rub the barnacles off. It is protected by the New Zealand government. The crew on one steamer shot at it & the report is that it has never visited that steamer since; I have not seen the fish myself.” However, the information from the June letter was not referring to the skeleton shipped to Strasbourg but instead to a famous ‘fish’ named Pelorus Jack that was actually not a beaked whale, but a Risso’s dolphin (Cowan 1912; Alpers 1960; McLintock 1966).

In the October letter, Dall describes trying to acquire the second skeleton, saying, “I am also trying to get a skeleton of *Sibbaldius sulphureus* the fish is 30 ft. long, but it is in a bad place so I am not certain that I can obtain the perfect skeleton.” However, by the time he wrote the June 1906 letter, Dall has apparently been able to obtain the skeleton as he writes, “I kept one fin of *Sibbaldius sulphureus*

in the flesh (there is no smell with it except a little oil) as I thought it would give a better idea how to place the bones.... I will have a good look again for the eyeball of *Sibbaldius sulphureus* which I buried." We know less of the third skeleton with only a short line in a July, 1906, shipping description reading, "incomplete skeleton of the supposed *?Epiodon NovaeZelandiae?* skull with ear drums in position".<sup>1</sup>

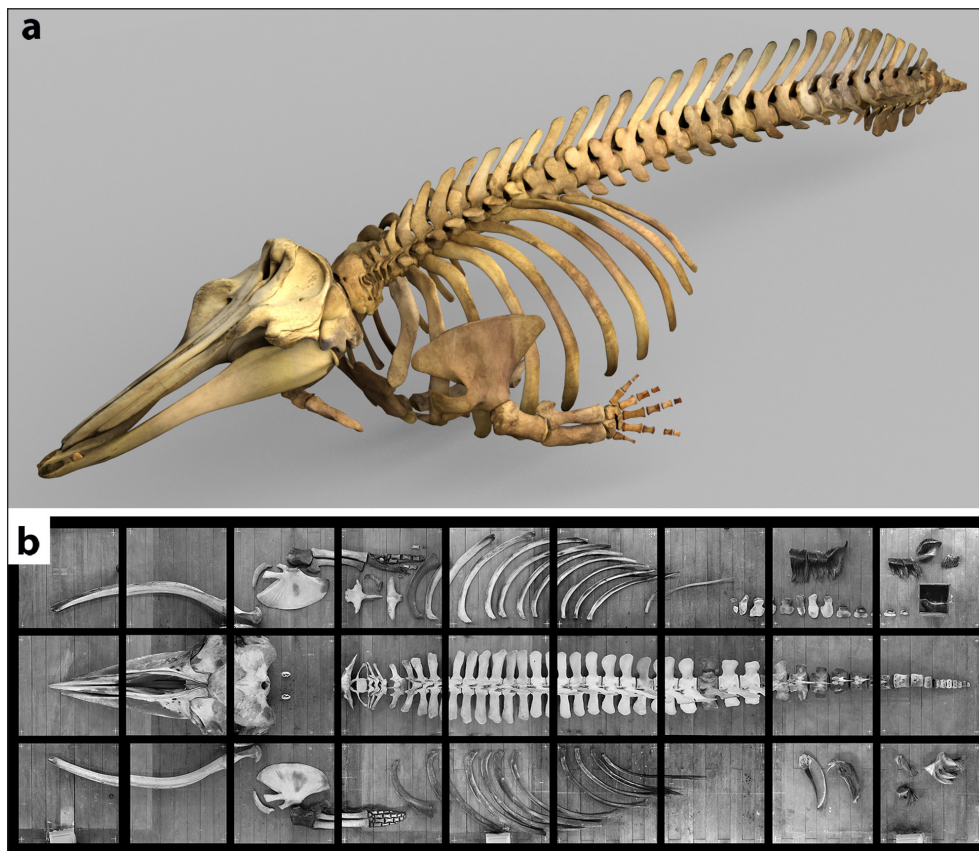
### 3.3. Osteological determinations and observations

Osteological analyses determined that four samples were from toothed whales and nine were from baleen whales (**Table 1**). Of the toothed whales, three partial skulls were easily identified as sperm whales (*Physeter macrocephalus*). MZS Mam11094 is a mandible with many teeth missing. MZS Mam11106 is a portion of a mandible with the anterior part sawed off. MZS Mam11119 is the cranium and upper jaw in two pieces that were formerly held together with large metal screws (**Figure 1a**). They all likely belong to adult individuals. In addition, one complete skeleton (MZS Mam11112) was identified as Arnoux's beaked whale (*Berardius arnuxii*). All bones were marked with 'II' and are consistent with being from the same individual. Only one tooth is missing from this skeleton (**Figure 2a**).

Of the nine baleen whales, only one could be satisfactorily determined using osteology to a specific species: MZS Mam11118, the baleen whale cranium in the entrance hall, was identified as right whale (*Eubalaena glacialis*) which

contradicts the *Balaena mysticetus* label (**Figure 1a**). MZS Mam11103 is half a mandible from a baleen whale which was formerly exhibited in the entrance hall as confirmed by previous curators and is referred to on inventory cards as *Balaena mysticetus*. It is 6.15 m in length, suggesting that if the whale was *Balaena mysticetus*, it was a large adult some 20 m long. This half mandible was further sawed in two halves when it was moved to the attic circa 1980 because it was too large and heavy to be moved as one piece. The split bone is covered with historical carved graffiti (words and letters), one of which is dated 1703. One of the incomplete skeletons (MZS Mam11113) was identified as likely corresponding to one individual and all bones are marked with a 'I'. Both MZS Mam11103 and the skeleton (MZS Mam11113) could be identified as baleen whale, but not to the genus level.

Four samples exhibit holes that likely correspond to a previous mounting structure: sample MZS Mam11104 is an incomplete skull; MZS Mam11105 includes eight mandible fragments; MZS Mam11114 is a single bone fragment; and MZS Mam11109 includes thirteen vertebrae and nine ribs. Osteological analysis showed that there are no bone duplications within these four samples, all are from a baleen whale, are from the same sized individual and have similar appearance. Although the species could not be determined, they were tentatively considered to be from one individual after osteological analysis. The final two samples are the complete skull (with the two



**Figure 2: Two reconstituted whale skeletons. (a)** 3D reconstruction of the skeleton of a *Berardius arnuxii* specimen (MZS Mam11112) as completed by Laetoli Production. **(b)** All elements of the skeleton of a minke whale (MZS Mam11110 and MZS Mam11111). Photo Vincent Chevillon.

first vertebrae, atlas and axis) of a baleen whale (MZS Mam11110) and the headless skeleton of a baleen whale (MZS Mam11111). All of the bones from the latter are marked with 'B'. The baleen is missing from the skull, but the tympanic bullae are in place. The skeleton comprises one fin which was dried with the flesh still attached and some of the vertebrae are covered with barnacles as if the bones had lain stranded in the intertidal zone before being recovered. In addition, there were two tympanic bullae (those wrapped in the New Zealand newspaper) and a box containing baleen plates, all marked with 'B' which were inventoried under the MZS Mam11111 identification. Although the bones for both MZS Mam11110 and MZS Mam11111 are still grimy with a yellowish greasy ooze and oxidizing on their surface, initial evaluation could not confirm that these bones were from one individual (**Figure 2b**). Osteological analysis indicated that the size and conformation could be consistent with this hypothesis; however the skull shape (MZS Mam11110) was close to that of a humpback whale (*Megaptera novaeangliae*) but the anterior limbs were not, and MZS Mam11111 was tentatively identified as blue whale (*Balaenoptera musculus*) despite some elements not supporting this identification.

### 3.4. ZooMS identifications

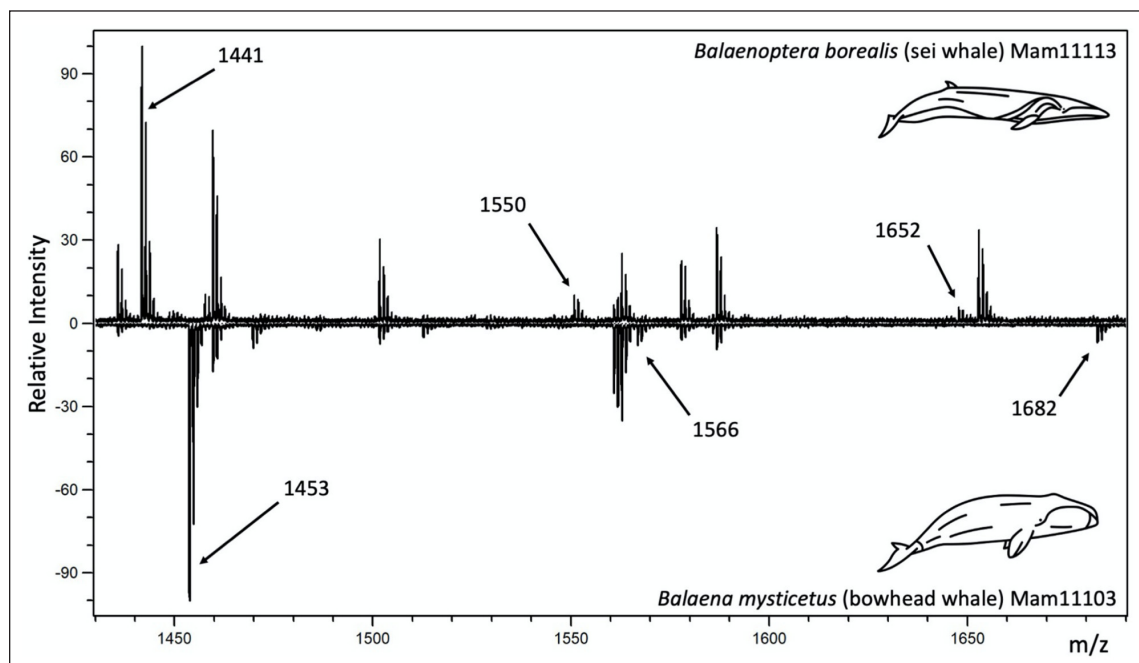
The nine published markers for ZooMS are able to separate the following species (species separated by a '/' indicate that these two species can be distinguished from all of the others, but have identical markers and therefore cannot be separated from each other using ZooMS): sperm (*Physeter catodon*), humpback (*Megaptera novaeangliae*), beluga (*Delphinapterus leucas*), gray (*Eschrichtius robustus*), fin (*Balaenoptera physalus*), blue (*Balaenoptera musculus*),

sei (*Balaenoptera borealis*), minke (*Balaenoptera acutorostrata/Balaenoptera bonaerensis*), right or bowhead (*Eubalaena glacialis/Balaena mysticetus*), bottlenose or Sowerby's (*Hyperoodon ampullatus/Mesoplodon bidens*). Identifications were possible for all 14 samples (**Table 1**, SI2 Table 2). Four of the samples each represented one sample number. Of these, two (MZS Mam11103 and MZS Mam11118) were identified as either right or bowhead whale which cannot be distinguished using ZooMS (**Figure 3**). The other two (MZS Mam11104 and MZS Mam11109) were identified using ZooMS as fin whale.

The other ten samples were from three sample numbers. MZS Mam11113 had samples taken from the skull and from a vertebra. Both of these samples were clearly identified by ZooMS as sei whale (**Figure 3**). MZS Mam11110 had samples taken from the skull and mandible. One of the samples was clearly identified as minke whale. The other sample was identified as either minke or sei whale because the sample was missing the one marker which distinguishes between these two species. MZS Mam11111 had six samples taken from vertebra 16, rib 5L, the left arm, the haemal arch, the sternum, and the scapula. All but one of these samples were identified uniquely as minke. The final sample could only be identified as either minke or sei because the marker that distinguishes between the two species was missing.

### 3.5. Combining identifications and reuniting individuals

Using a combination of written information, osteological analysis, and ZooMS investigation, we were able to identify the species of all of the 13 specimens (**Table 1**). Four of the samples were uniquely identified by classical



**Figure 3: ZooMS identification.** Example whale spectra from two individuals, MZS Mam11103 (*Balaena mysticetus*) and MZS Mam11113 (*Balaenoptera borealis*), indicating the three key markers that are used to distinguish the whale species. Marker B has variants at m/z 1441 and 1453. Marker C has variants at m/z 1550 and 1566. Marker P2 has variants at m/z 1652 and 1682 (Buckley et al. 2014). Whale images by Tom Fricker from thenounproject.org.



osteology (MZS Mam11094, Mam11106, Mam11119, and Mam11112). Of these four specimens, two had paper inventory cards with the correct species (MZS Mam11094 and Mam11119). The other two had no information associated with the bones. In addition, it is likely that MZS Mam11112 is one of the whales described in the letters from Dall. Indeed, he mentions a *Berardius arnuxii* complete skeleton missing one tooth. This is consistent with the species identification and osteological analysis of MZS Mam11112, meaning this skeleton was obtained from Para Para Beach in Collingwood New Zealand, in January 1905.

The remaining samples were identified using both osteological analysis and ZooMS. In two cases, ZooMS was not able to uniquely identify the samples, but the osteology clarified which of the two species was correct. Both MZS Mam11103 and Mam11118 were identified through ZooMS as either right or bowhead whale. Osteology identified MZS Mam11103 as bowhead whale in agreement with the paper inventory card. Based on the dated graffiti on the bone, this specimen likely pre-dates 1703. MZS Mam11118 was identified by osteology as a right whale which contradicts the display tag from the 1980s. It is likely that this skull was on display since the late 1890s and as such, likely dates to that period, if not before.

In three cases, ZooMS was able to provide more specificity than osteology. MZS Mam11113 could only be identified as Mysticeti and lacked historical records. ZooMS identified the sample as sei whale. Both MZS Mam11104 and Mam11109 could also only be recognized as Mysticeti by osteology. They were both identified as fin whale using ZooMS. This corresponds to the label associated with MZS Mam11109, which indicates that it is of the genus "*Balaenoptera*". It is likely that MZS Mam11104 and Mam11109 are from the same individual because of the bone features mentioned above. This specimen certainly also includes MZS Mam11105 and Mam11114; the latter has an attached label stating "*Balaena mysticetus*; zum Schädel gehörig" and belongs to the skull, which could well be MZS Mam11104. However, this means that the species information on the label is incorrect.

ZooMS contradicted the osteological identifications in two cases. MZS Mam11110 was identified as humpback whale and MZS Mam11111 was tentatively identified as blue whale osteologically, but ZooMS indicated clearly from multiple samples that both the skull and skeleton belonged to a minke whale. Both the skull and skeleton were difficult to identify given the absence of an authenticated reference dataset for Cetacea both in the descriptions available and the reference specimens in other museums. Given the ZooMS identifications and the similarity in the bone features, size, and condition, it is likely that MZS Mam11110 and Mam11111 are from one individual. This individual clearly fits with the description of one of the skeletons sent by Dall in 1906, namely the specimen with one fin still in the flesh (**Figure 2b**).

#### 4. Discussion

Museum collections very often harbour precious natural history specimens that unfortunately have lost their associated information over time or have been provided with erroneous or out of date taxonomic identifications. Yet,

it is nonetheless important to characterize them as accurately as possible, particularly when entering collections into digital inventories and databases. ZooMS is emerging as a cost-effective biomolecular method for museums to fulfil this task, as it requires very little material (10–30 mg of bone) and can be performed non-destructively even on small bones (such as from fish or rodents). For the whale bones described here, it proved a highly efficient and reliable technique that, together with classical osteological investigations and historical record searching, allowed us to reconstruct the species and partial histories for the 57 bones or sets of bones belonging to whales from the MZS. Of the 13 bones or sets of bones which had questionable identities during the initial screening, all were identified to the species level using the combined approach. As anticipated, not all of the historical labels were accurate: most notably MZS Mam11118, which was displayed in the entrance hall for years as a bowhead whale, was found rather to be a right whale. In addition, we were able to reunite bones from two different individuals. MZS Mam11104, Mam11105, Mam11109, and MZS Mam11114 are likely from the same individual based on the osteology and the marks on the bones from past display. They were confirmed with ZooMS to all be from the same species. MZS Mam11110 and Mam11111, while showing similar texture and deriving from the same sized individual, were tentatively identified by comparative osteology as being the skeleton and skull of different species and therefore not from the same individual. ZooMS was able to confirm that they were in fact from the same species and therefore likely from the same individual given the other osteological information.

In addition to the identifications, we were able to link three of the bone sets to more specific information about their context. MZS Mam11103 is remarkable – at 6.15 m long and 150 kg, it is clear that this half mandible came from a very large individual (estimated to have been c. 20 m long). The graffiti dates the bone to not later than 1703, suggesting it is one of the oldest items in the MZS collections. It is possibly referred to by Frédéric Piton in 1855 (Piton 1855) when he describes bones given to the city in 1577 by a carnival showman after they had been exhibited during the city fair and were subsequently displayed at the Arsenal of the City of Strasbourg (J.P. Rieb, pers. comm.). To confirm the 16<sup>th</sup>-century origin of the bone, radiocarbon dating could be performed in the future.

The museum purchased three skeletons from James Dall that were sent from New Zealand in 1906. We were able to locate and reconstruct two of those skeletons. MZS Mam11112 is the complete skeleton of the Arnoux's beaked whale that washed up on Para Para Beach in January 1905. It is very well preserved and only missing one tooth, as noted by Dall prior to purchase. Every bone has been digitized, allowing for a 3D reconstruction freely available through the Vertebrate application at Laetoli Production (**Figure 2a** and <https://www.laetoli-production.fr/vertebrates>). Arnoux's beaked whales are members of the four-toothed whales (*Berardius*), which are the largest whales in the family Ziphiidae, growing easily to over 11 m as adults. Reconstruction of the length indicates that this individual was around 8.5 m long at the time of death.

The minke whale skeletal parts (MZS Mam11110 and Mam11111) are likely from a single individual, described as a *Sibbaldius sulphureus* carcass that washed up sometime before October 1905 and which Dall allowed to degrade on the shore, aside from one fin which he kept fleshed. The dried fin is still associated with this individual. Since it was found on a beach in New Zealand, there is a reasonable chance that it is an Antarctic minke whale or southern minke whale (*Balaenoptera bonaerensis*) rather than northern minke whale (*Balaenoptera acutorostrata*), which does not range in southern seas. But since whales do sometimes venture out of their distribution range (Rosel et al. 2016), the only way to be certain of its identity would be to conduct a DNA analysis. It should also be noted that according to our initial osteological comparisons, the skull did not show marked affinities with that of a northern minke whale specimen displayed at the MNHN. Minke whales are rather small, with an average length of 7–8 m; our specimen is probably in the higher range of these values, although it is difficult to calculate a good estimate on a disconnected skeleton, where some bones may even be missing (nb: 11 left vs 12 right ribs). There is also the remaining problem that there are four tympanic bullae associated with this individual (two in place and two wrapped separately with the same label), an issue that remains unresolved. It seems obvious that the second pair derive from a different animal, but we have no way to identify this individual.

Para Para Beach is in Golden Bay (north of South Island), which is a resting area for migrating whales and dolphins. It is close to Farewell Spit, a narrow sand spit at the northern end of the bay, which functions as a natural trap and where whale stranding has been a common occurrence throughout history (Betty et al. 2020; Brabyn and McLean 1992; Brabyn 1991). These individuals may have been stranded and given both the descriptions by Dall and the barnacles attached to the vertebra of MZS Mam11111, they were likely skeletonized by the sea. He may therefore have collected items from other remains on the beach which may account for the additional tympanic bullae (see Meister et al. 2020). Sadly, we were unable to locate the third, incomplete, skeleton sent by Dall in 1906 and which was supposed to be of *Epiodon novaezelandiae* (proposed synonym *Ziphius cavirostris*).

We were also unable to connect any specimen to the entry in the catalogue and register that mentions a *Balaenoptera Sibbaldii* skeleton from Vardo. This likely refers to a *Balaenoptera borealis* or sei whale, although the reference to teeth is confusing because sei whales are baleen and not toothed whales. While MZS Mam11113 is the partial skeleton of a sei whale, we do not feel there is enough additional information to connect this individual to the reference. The unfortunate reality is that sometimes specimens vanish from collections.

## 5. Conclusion

Recent years have seen an explosion of research attempting to better understand the biodiversity of the oceans, particularly as many large marine dwelling species such as whales and sharks are threatened or endangered due to anthropogenic effects on the ocean environment and cli-

mate change (Albouy et al. 2017, 2020; Gray and Kennelly 2018; Avila, Kaschner, and Dormann 2018; Baker et al. 2016; Forney et al. 2017). Historical collections provide a valuable resource in this endeavour (Barilaro 2018; McLean et al. 2016; Pyenson 2018; Pyke and Ehrlich 2010; Johnson et al. 2011). Not only do they serve an important purpose in public displays and educational resources (Cook et al. 2016; Ballard et al. 2017), they also provide reference collections for morphological analyses of bones (LeFebvre and Sharpe 2018; Lyman 2010) and study samples for biomolecular techniques (Eisenmann et al. 2016; Harmon, Littlewood, and Wood 2019; Nganvongpanit et al. 2017; Schmitt et al. 2018). Since a reference osteological database for whales is lacking in completeness, with guidebooks insufficiently authenticated and not covering the extent of diversity within or hybridizations between species (Bérubé and Palsbøll 2018), authenticating museum specimens can help provide accurate reference collections for researchers, especially given the potential today for 3D scanning and printing (Nobles, Çakırlar, and Svetachov 2019). In addition, museum collections can be used to reconstruct food webs, migration patterns, disease outbreaks, health, and population size and structure from the historical period, which is all the more relevant to understanding healthy whale populations in these key marine species. However, in order for these collections to be broadly used, catalogues must be digitized, and collections must be authenticated which is a long and sometimes difficult process. As we have shown here, a multidisciplinary combination of approaches provides a relatively low-cost way to improve the accuracy of available information about museum collections so that they can be broadly accessible for education and research.

## Data Accessibility Statement

Raw data is provided in the supplementary tables and at the following digital locations.

Collection Inventory on GBIF: <https://www.gbif.org/fr/>.

Dall's Letters: <https://archipels.org/atlas/lettres-oderlin>.

3D Digital Reproduction Mam11112: <https://www.laetoli-production.fr/vertebrates>.

ZooMS Spectra: Zenodo Record Number: 3746000 doi: 10.5281/zenodo.3746000.

## Note

<sup>1</sup> The letters can be found at <https://archipels.org/atlas/lettres-oderlin>.

## Additional Files

The additional files for this article can be found as follows:

- **Supplementary file 1.** Images of ZooMS spectra. DOI: <https://doi.org/10.5334/jcms.196.s1>
- **Supplementary file 2.** Supplementary tables in excel format. DOI: <https://doi.org/10.5334/jcms.196.s2>

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### Competing Interests

The authors have no competing interests to declare.

### Author Contribution

Antoine Wagner and Kristine Korzow Richter contributed equally to the paper.

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