Journal of Archaeological Science: Reports 15 (2017) 262–271

Contents lists available at ScienceDirect

Journal of Archaeological Science: Reports

journal homepage: www.elsevier.com/locate/jasrep

Genomic and proteomic identification of Late Holocene remains: Setting baselines for Black Sea odontocetes

Vincent Biard[a,*], Pavel Gol'din[b], Elena Gladilina[b,c], Karina Vishnyakova[b,c], Krista McGrath[d], Filipe G. Vieira[a], Nathan Wales[a], Michael C. Fontaine[e], Camilla Speller[d], Morten Tange Olsen[a,**]

[a] Section for Evolutionary Genomics, Natural History Museum of Denmark, University of Copenhagen, Øster Voldgade 5-7, 1350 Copenhagen K, Denmark
[b] Schmalhausen Institute of Zoology, National Academy of Sciences of Ukraine, 15 Bogdan Khmelnytskyi Street, Kiev 01601, Ukraine
[c] Ukrainian Scientific Centre of Ecology of the Sea, 89 Frantsuzsky Blvrd., Odessa 65009, Ukraine
[d] BioArCh, Department of Archaeology, University of York, Environment Building, York, North Yorkshire YO10 5DD, UK
[e] Groningen Institute for Evolutionary Life Sciences (GELIFES), University of Groningen, PO Box 11103 CC, Groningen, The Netherlands

ARTICLE INFO

Keywords:
Species identification
Mixed assemblage
Odontocete
ZooMS
Shotgun sequencing
Sanger sequencing
Mitogenome

ABSTRACT

A critical challenge of the 21st century is to understand and minimise the effects of human activities on biodiversity. Cetaceans are a prime concern in biodiversity research, as many species still suffer from human impacts despite decades of management and conservation efforts. Zooarchaeology constitutes a valuable approach for informing conservation and management decisions by providing baseline information on the past distribution and human uses of species. However, traditional morphological species identification of mixed assemblage bones can be challenging, particularly in the case of cetaceans. To address this issue, we applied and evaluated the performance of three biomolecular approaches – Sanger sequencing, shotgun sequencing and collagen peptide fingerprinting (ZooMS) – for species identification in a mixed assemblage of 800 to 1600 years old odontocete (toothed whale) samples from the site of Chersonesus in Crimea, Ukraine. We found that ZooMS allowed for identification to the taxonomic level for 28 of our 30 samples (> 90%), identifying them as either "porpoise" or "dolphin", and approximately half of those samples could be further identified to species level with the shotgun sequencing approach. In addition, shotgun sequencing produced several complete ancient odontocete mitogenomes and auxiliary nuclear genomic data for further exploration in a population genetic context. In contrast, both morphological identification and Sanger sequencing lacked taxonomic resolution and/or resulted in misclassification of samples. We found that the combination of ZooMS and shotgun sequencing provides a powerful tool in zooarchaeology, and here allowed for a deeper understanding of past marine resource use and its implication for current management and conservation of Black Sea odontocetes.

1. Introduction

One of the critical challenges of the 21st century is to investigate and reduce the negative effects of human activities on the Earth's biodiversity. To address these issues, detailed baseline information on the ecology and evolution of plant and animal populations and species is required, including information on past abundance, distribution, species life history or phenology, as well as historic and prehistoric human resource use, context and impacts. While this is true for much of Earth's biota, cetaceans constitute an iconic group where many species and populations, despite decades of management and conservation efforts, still suffer from past and present human impacts, such as overexploitation, habitat alterations, bycatch, and ocean noise (Schipper et al., 2008; Pimm et al., 2014).

Zooarchaeology is particularly suited to provide such baseline information, as this approach documents a broad range of direct material evidences of animals, including their abundance, distribution, diversity, population structure and individual traits, as well as long-term changes of these parameters (Steadman, 1995; Dietl et al., 2015; Hofman et al., 2015). However, in the case of cetaceans, accurate identification of species constitutes a special problem. On many archaeological sites, cetacean assemblages contain multiple species (Mulville, 2002), which are represented by only incomplete fragments of postcranial bones and often are considered to be non-diagnosable by morphological methods

* Correspondence to: V. Biard, 4 Rue Roger Senturenne, D103, 33140 Villenave d'Ornon, France.
** Correspondence to: M.T. Olsen, Section for Evolutionary Genomics, Natural History Museum of Denmark, University of Copenhagen, Øster Voldgade 5-7, 1350 Copenhagen K, Denmark.
E-mail addresses: vincentbiard33@gmail.com (V. Biard), morten.olsen@snm.ku.dk (M.T. Olsen).

http://dx.doi.org/10.1016/j.jasrep.2017.07.008
Received 30 March 2017; Received in revised form 4 July 2017; Accepted 8 July 2017

(Mulville, 2002; Amundsen et al., 2013). For example, isolated vertebrae of *Delphinus* sp. are hardly discriminated from *Stenella* sp., small *Tursiops* sp. or *Steno* sp. Furthermore, in an assemblage containing kitchen refuse of *Delphinus* sp., *Stenella* sp. and *Tursiops* sp., most of postcranial fragments were unidentified, unlike well informative cranial and, in particular, dental remains (Cooke et al., 2016). In cetaceans, caudal vertebrae (comprising 35–50% of all vertebrae, depending on the species) and vertebral centra with missing processes are especially difficult for diagnostics. This may also be true for other mammalian groups, but many terrestrial mammalian taxa are often represented by a single species in each assemblage or a few species of different size categories and, thus, are more easily discriminated (Dunnell, 1971; Grayson, 1984).

The ability to extract and analyse ancient DNA (aDNA) from subfossil material have greatly contributed to the resolution of this problem (Willerslev and Cooper, 2005), allowing for determining the species identity of e.g. historical whale remains from whaling stations at South Georgia (Lindqvist et al., 2009; Sremba et al., 2010). Moreover, although driven primarily by research in human (Briggs et al., 2009; Rasmussen et al., 2010; Allentoft et al., 2015) and terrestrial megafauna evolution (Gilbert et al., 2008; Lorenzen et al., 2011; Dabney et al., 2013a), several studies have utilized aDNA to assess the evolutionary and demographic history of cetaceans (see Foote et al., 2012a). For instance, McLeod et al. (2012) have examined the demographic history of bowhead whales (*Balaena mysticetus*) in the Canadian Arctic using mitochondrial DNA fragment, and have revealed a population expansion over the past 30,000 years BP. Similar historic inference have been made from Eastern Atlantic bowhead whales (Foote et al., 2013), as well as common bottlenose dolphin (*Tursiops truncatus*) (Nichols et al., 2007), killer whales (*Orcinus orca*) (Foote et al., 2009) and North Atlantic right whales (*Eubalaena glacialis*) (McLeod et al., 2010).

To date, most molecular approaches for ancient cetacean species determination have been based on Sanger sequencing of short fragments of mitochondrial genes such as cytochrome-b (CytB), ribosomal 12S or cytochrome oxidase-I (COI). However, for highly degraded material, sufficient DNA template may not be preserved, or this approach might not yield sufficient coverage and resolution for species distinction – in particular if these are from closely related species (Goldstein and DeSalle, 2011). Moreover, the focus on a single short fragment often limits additional population genetic inference of evolutionary and demographic trajectories, as well as associated historical anthropogenic and climatic impacts. The recent development of peptide mass fingerprinting of bone collagen (ZooMS), where species identification can be accomplished by comparing ancient peptide fingerprints to modern reference databases, allows for analysis of degraded ancient material otherwise suboptimal for DNA analysis (Buckley et al., 2009; Welker et al., 2015). However, due to the relatively slow rate of evolution within the collagen chains, taxonomic resolution is often limited to the genus or even family level. Studies combining analysis of aDNA Sanger sequencing and collagen targeting have successfully been applied to a few diverse assemblages of baleen whales (Buckley et al., 2014; Evans et al., 2016; Speller et al., 2016), however for some species and materials, even the combined use of these methods may not provide sufficient resolution for species identification. By generating massive amounts of data across the genome, short-read DNA “shotgun” sequencing techniques may circumvent the obstacles of aDNA fragmentation and taxonomic resolution, as well as provide additional data for population genetic inference of demography and phylogeography (Leonardi et al., 2016). Thus, the approach has a great potential to become the gold standard for species identification of ancient material. However to date the applicability of shotgun sequencing relative to Sanger sequencing and collagen profiling have not been evaluated.

All three odontocete populations inhabiting the Black Sea, the common bottlenose dolphin (*Tursiops truncatus*), the short-beaked common dolphin (*Delphinus delphis*) and the harbour porpoise (*Phocoena phocoena*), though of widespread species, are recognized as separate subspecies and of special management and conservation concern. The IUCN has classified the Black Sea harbour porpoise (*P. p. relicta*) and the Black Sea bottlenose dolphin (*T. t. ponticus*) as endangered (Birkun and Frantzis, 2008; Birkun, 2012) and the Black Sea common dolphin (*D. d. ponticus*) as vulnerable (Birkun, 2008). These have all been severely depleted by decades of extensive hunting (Kleinenberg, 1956; Birkun, 2002a), and are now affected by fisheries bycatch and consequences of ctenophore *Mnemiopsis leidyi* invasion, undermining their trophic base (Bushuev, 2000; Vishnyakova and Gol'din, 2015b). The common bottlenose dolphin (Viaud-Martinez et al., 2008; Moura et al., 2013) and short-beaked common dolphin (Amaral et al., 2007) both appear to have colonised the Black Sea a few thousand years ago. Similarly, it has been suggested that a relict harbour porpoise population in the Eastern Mediterranean founded the Black Sea population a few thousand years after the reconnection of the two basins, probably tracking suitable habitats (Fontaine et al., 2012; Fontaine et al., 2014; Fontaine, 2016). Moreover, in contrast to the two other odontocete species present in the basin, the Black Sea harbour porpoise is unique in its isolation to other populations situated in the Atlantic Ocean, around 4000 km apart. Its absence from the Mediterranean Sea, already noted by Aristotle (350 BCE) (Frantzis et al., 2001), might be the consequence of changing oceanic conditions, notably warming temperatures and low productivity of Mediterranean ecosystems (Thunell et al., 1977; Thunell, 1979; Fontaine, 2016). Thus isolated, the Black Sea harbour porpoise has evolved distinct morphological and genetic characteristics (Viaud-Martínez et al., 2007; Galatius and Gol'din, 2011), and additional sub-structuring might even exist among the different water bodies of the Black Sea region (Gol'din, 2004; Gol'din and Vishnyakova, 2016), although this question requires further investigations.

Here we tested the applicability of different species identification methods on a mixed assemblage of odontocete (toothed whale) zooarchaeological remains excavated at the site of Chersonesus on the Black Sea coast of Crimea, Ukraine. Specifically, we i) compared and evaluated the performance of four approaches: morphology, Sanger sequencing, collagen peptide mass fingerprinting (ZooMS), and shotgun sequencing of short-read DNA; and ii) discuss our findings in the context of historic marine resource use, and in relation to setting baselines for contemporary conservation and management schemes in the region. To our knowledge this is one of the first studies to utilize the full extent of recent genomic and proteomic techniques to identify and analyse highly degraded ancient odontocete material. Importantly, although the present focus is on odontocetes, our findings should apply to other organisms and study systems.

2. Materials & methods

2.1. Study site

Chersonesus (also known as Chersonesus Taurica or Tauric Chersonesus = Chersonese) is located on the Black Sea coast in the southern Crimea (Supplementary Fig. S1). It was the greatest city, trade and cultural centre of the northern Black Sea region during the Hellenistic, Roman and Byzantine ages between 2400 and 600 years BP (Strabo, 1929; Porphyrogenitus, 1993; Carter and Mack, 2003). Founded by Greeks from Asia Minor, it was populated by colonists and visited by traders from various Black Sea and Mediterranean localities, and thus, the archaeology of the city shows a great variety of regional economic and cultural practices (Kadeev, 1970; Kadeev and Sorochan, 1989). In particular, marine fisheries and seafood played an important role in economy and diet of the Chersonesus population, and diverse marine fauna was reported from zooarchaeological evidence, as well as from art representations and some descriptive sources (Semenov-Zuser, 1947; Kadeev, 1970; Højte, 2005; Morales et al., 2007).