Use of next-generation molecular tools in archaeological neotropical deer sample analysis

Federica Moreno [a], Gonzalo Figueiro [b], Natalia Mannise [c], Andrés Iriarte [d,e], Susana González [c,f], José Mauricio Barbanti Duarte [g], Mariana Cosse [c,*]

[a] Programa de Investigación Antropo-Arqueológica, DICYT-MEC, Uruguay
[b] Departamento de Antropología Biológica, FHCE-Udelar, Uruguay
[c] Departamento de Biodiversidad y Genética, IIBCE-MEC, Uruguay
[d] Departamento de Genómica, IIBCE-MEC, Uruguay
[e] Departamento de Desarrollo Biotecnológico, Instituto de Higiene, Facultad de Medicina, Udelar, Uruguay
[f] Sección Genética Evolutiva Facultad de Ciencias, Udelar, Uruguay
[g] Núcleo de Pesquisa e Conservação de Cervídeos, Departamento de Zootecnia/Universidade Estadual Paulista Jaboticabal – SP-, Brazil

ARTICLE INFO

ABSTRACT

Taxonomic identity and genetic variation of three bone samples from a late Holocene earth mound in the eastern Uruguayan lowlands were analyzed. Samples were initially identified by morphology as being from pampas deer (*Ozotoceros bezoarticus*), a middle-sized cervid that occupies open environments forming herds of up to 50 animals. Prehistoric human communities may have developed herd management systems for this species. The aim of this study was to evaluate usefulness of a Next Generation Sequencing (NGS) strategy using pooled samples to explore genetic relationships between specimens. Two mitochondrial *D-loop* haplotypes were found which surprisingly identifies another deer species (*Mazama gouazoubira*). Our findings confirm that NGS is a powerful tool for taxonomic identification of ancient Uruguayan samples, also indicating that *M. gouazoubira* may be underrepresented in the zooarchaeological record of eastern Uruguay. Finally, since both species have different niches and behaviors it is necessary to formulate new questions in order to understand prehistoric management of Neotropical deer involving larger sample sizes and analysis of other genetic markers.

© 2016 Elsevier Ltd. All rights reserved.

1. Introduction

The Uruguayan lowlands located in southeastern South America, were occupied by mound building groups dating back from the mid-Holocene until the European conquest. The mounds are known locally as "*Cerritos de Indios*" (López Mazz, 2001; Bracco, 2006). Within this region archaeological studies indicate that diverse socio-economic features were developed by these groups, including domestication of plants and horticulture, a trend towards sedentarization and social complexity, villages and public architecture, territorial control and formal cemeteries (López Mazz, 2001; Bracco, 2006; Iriarte, 2006). Paleoenvironmental studies suggest the beginning for establishing these villages and activities initiated ca. 2500 years BP (Bracco et al., 2005a; Bracco et al., 2005b; Del Puerto et al., 2011).

Zooarchaeological studies of *cerritos* suggest the exploitation of cervids including pampas deer (*Ozotoceros bezoarticus*), marsh deer (*Blastocerus dichotomus*) and in a lesser degree brown brocket deer (*Mazama gouazoubira*), small and medium-sized rodents (*Myocastor*

coypus, *Cavia* sp. and *Hydrochoeris hydrochaeris*), ñandú (*Rhea americana*), pinnipeds (*Otaria flavescens* and *Arctocephalus australis*), and several fish species (siluriformes, *Pogonias cromis* and *Micropogonias furnieri*). Fragmentation rates of the assemblages are around 90%. As such, 70% of taxonomic determinations of remains cannot be made beyond the class level in zooarchaeological collections (Pintos, 2000; Iriarte, 2006; Moreno, 2014).

In spite of the variety of species found in assemblages, Pintos (2001) proposed that the base of the animal economy were cervids and pinnipeds. Within the ungulates, the pampas deer (*O. bezoarticus*), a middle-sized cervid that occupies open environments forming herds of up to 50 individuals (Cosse and González, 2013), was found most frequently (Moreno, 2014). The social complexity proposed for the archaeological groups who built these *cerritos* allows for the formulation of hypotheses on how they controlled and exploited wild animals such as the pampas deer (*O. bezoarticus*), as a stage in the intensification process going from hunting to domestication (Vigne, 2011). One form of control could imply the exclusive use of the resources within a given territory, possibly including herd management.

The control of wild animals does not necessarily imply or result in morphological changes visible at an osteological level (Vigne, 2011).

* Corresponding author.
E-mail address: mcosse@iibce.edu.uy (M. Cosse).

Testing hypotheses on animal control methods by ancient people relies on development of alternative tools such as those based on genetics. Ancient DNA research in archaeological faunal remains is focused on answering questions related to the domestication and management of various species (Leonard et al., 2002; Cai et al., 2007; Deguilloux et al., 2009; Røed et al., 2011; Sykes et al., 2011; Gravlund et al., 2012; Thalmann et al., 2013). In addition, the recent development of Next Generation Sequencing (NGS) and its application to the taxonomic identification of bulk bone (pooled samples) offers a cost-effective methodology to deal with this challenge (Murray et al., 2013). This approach could be a powerful tool to understand the relationship between human and animal communities in Uruguayan prehistory.

The goal of this study was to evaluate the NGS methodology with pooled samples, to obtain highly informative genetic data from cervid archaeological remains recovered from excavations in eastern Uruguay.

2. Materials and methods

2.1. The site

The analyzed remains were recovered from the Ch2D01 archaeological site in the *bañado* (wetlands) of San Miguel, Rocha, Uruguay (Fig. 1). The site has two earth mounds (named A and B), with an occupation ranging from 2090 ± 90 years BP (KR139) to 220 ± 50 years BP (URU0014) (Bracco, 2006). The site has an extensive zooarchaeological record, and it is very well preserved in comparison to other assemblages excavated in the region (Moreno, 2014).

Bone fragments of three specimens recovered from the central excavation in the mound A (named IA) were processed. These samples were morphologically identified as pampas deer (*O. bezoarticus*) (Fig. 2) and dated at ca. 1300 years BP through stratigraphic association. Molecular studies of human remains excavated at the site yielded good results
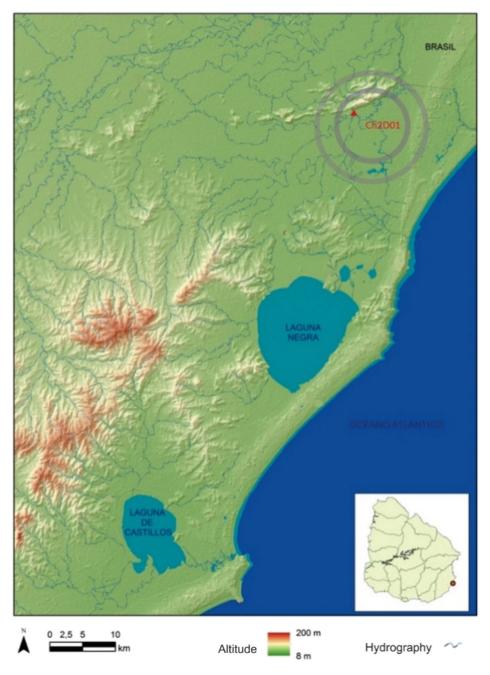

Fig. 1. Location of the Ch2D01 archaeological site in southeastern Uruguay.