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Morphometric analysis of modern faeces as a tool to identify artiodactyls' coprolites

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ABSTRACT

The identification of the zoological origin of coprolites is important to infer paleoecological characteristics of ancient wildlife. The aim of this study was to design a reliable tool to distinguish coprolites of artiodactyls collected from archaeological sites of Patagonia, Argentina. A reference collection of modern droppings of large size artiodactyls of Patagonia, *Lama guanicoe* (guanaco) and *Hippocamelus bisulcus* (huemul), was also studied. Generalized discriminant analysis using shape and size attributes was applied to 538 pellets ($n_{guanaco} = 240$, $n_{huemul} = 298$). Faecal material was photographed and multiple predictor variables were calculated by the Image J (Java 1.6.0_12) Program. Coprolites attributable to camelids by paleoparasitological analyses were also classified by the discriminant classification function obtained from the reference collection. Results indicated that pellet size and shape were able to discriminate droppings of guanaco from those of huemul (Pillai's trace = 0.93, $p = 0.0001$). The accuracy of the classification function was 100%, with all the modern faeces of guanaco and huemul correctly classified. The accuracy for the identification of camelid coprolites was 100%. This is the first study that used a discriminant classification function of modern dung to identify coprolites. This research also provides information for its potential application to other archaeological deposits of dung in order to confirm the zoological origin of samples.

1. Introduction

The study of coprolites and their contents provides diverse information useful to infer paleoecological conditions of ancient fauna and humans (Putman, 1984; Chame, 2003; Reinhard and Bryant, 2008), such as parasitic infections, health conditions, potential zoonoses, paleopharmacology, behavior, diet, paleoenvironment, seasonality of the occupation of the sites and use of resources, among others (Reinhard et al., 1991; Araújo et al., 2000; Chin, 2002; Dittmar and Teegen, 2003; Reinhard and Bryant, 2008). The presence of coprolite deposits can be indicative of the use of the site for animal husbandry (Linseele et al., 2010), and coprolites are often the only evidence of species that are not represented among bone remains (Linseele et al., 2010, 2013; Riemer, 2011). Despite this, coprolites have often been ignored and are rarely reported in archaeological research (Linseele et al., 2010, 2013; Riemer, 2011).

One of the most important challenging aspects of archaeological dung studies is the determination of the zoological origin of the samples. Erroneous identification of coprolites can result in skewed data (Farrell et al., 2000; Chin, 2002; Davison et al., 2002; Chame, 2003; Prugh and Ritland, 2005). Coprolite identification implies the observation of macroscopic features, such as shape and size, and contents (Reinhard and Bryant, 1992; Chame, 2003; Bryant and Dean, 2006).

Archaeological sites of Patagonia harbored small, ovoid coprolites attributable to artiodactyls, whose species identification by initial observation of faeces is difficult to achieve. Among modern large size artiodactyls of Patagonia are *Hippocamelus bisulcus* (huemul) and *Lama guanicoe* (guanaco), the main alimentary resources for human hunter–gathered populations during the Holocene (Miotti and Salemme, 1999; De Nigris, 2007).

The aim of this study was to design a reliable tool to distinguish coprolites of large artiodactyls collected from Patagonian archaeological sites. For this purpose, a reference collection of modern droppings of guanaco and huemul was studied. The goal was to determine if the General Discriminant Analysis applied to shape and size features of modern faeces can be used to identify coprolites of artiodactyls.

Coprolite identification was achieved by the combination of paleoparasitological and external feature evidence, in order to

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assess the reliability of identification of coprolites by the classification function obtained from the recent dung reference collection.

2. Regional setting

2.1. Perito Moreno National Park

Perito Moreno National Park (PMNP) is located in the southern part of the Cordillera de los Andes, Santa Cruz Province (47° 40′S, 72° 30′W). The climate is temperate-cold in summer and freezing during winter. As a consequence of the marked increases in rainfall from east to west (EW), the vegetation varies from steppe to the formation of dense forest. At present, the only South American camelid present in the PNPM is *L. guanicoe*.

2.2. Los Alerces National Park

Los Alerces National Park (LANP) is located in the Futaleufú Department, northwest Chubut province (42°50′S, 71°52′ W). It is included in a lake system that stems from two streams. The climate is temperate-cold wet. Rainfall is present as a sharp EW gradient over a vast semi-arid space. From the phytogeographical point of view, the park is in line with the subantarctic forests. Among wild herbivores, LANP is home to an abundant population of pudú, the dwarf deer (*Pudu puda*), and established populations of huemul (*H. bisulcus*). By means of the "Huemul Conservation Programme", intensive surveys have recently been conducted in the park.

3. Materials and methods

3.1. Recent dung for reference collection

A reference collection of modern droppings of camelid and a large native cervid inhabiting the studied area was performed to identify artiodactyl coprolites collected from Patagonian archaeological sites. Two wild species of camelids live in South America at present: guanacos (*L. guanicoe*) and vicuñas (*Vicugna vicugna*). Only the guanaco inhabits Patagonia. A large native cervid of Patagonia is the huemul.

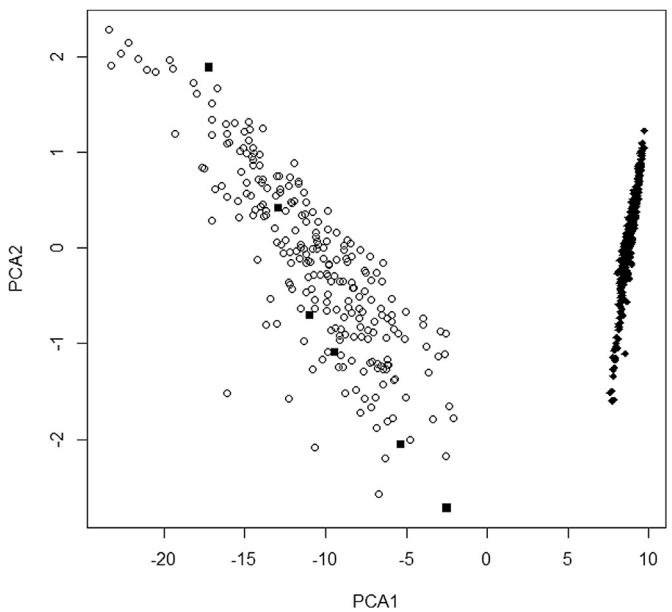

Fig. 1. PCA ordination for guanaco and huemul pellets dimensions. PCA1: Principal component axis 1; PCA2: Principal component axis 2. ◆: modern guanaco faeces; modern huemul faeces; ■: camelid coprolites.

For the reference collection, faecal samples of wild guanaco and huemul were collected from PMNP and LANP respectively, during 2010–2011, and 538 pellets ($n_{guanaco} = 240$, $n_{huemul} = 298$) were examined. Pellets were desiccated in an oven at 25 °C for 3 days, until their weight did not vary.

3.2. Coprolites

Coprolites ($n = 6$) were collected from different layers of the archaeological site Cerro Casa de Piedra, cave 7 (CCP7) (Table 1). Cerro Casa de Piedra is a volcanic outcrop located in PMNP, exhibiting a set of rock shelters and caves (Civalero and Aschero, 2003; Civalero and Franco, 2003). Cerro Casa de Piedra, cave 7 shows a stratigraphic sequence of 19 levels with human occupation between *ca.* 9700 ± 100 and 3600 ± 70 BP (Table 1) (Aschero, 1996; Civalero and Aschero, 2003). Coprolites examined in the present research were identified by Taglioretti (2008) and Taglioretti et al. (2009) as camelids by the presence of *Eimeria macusaniensis* oocysts, a specific parasite of South American camelids.

Table 1
Camelids coprolites collected from the archaeological site Cerro Casa de Piedra, cave 7 (CCP7).

N	Archaeological layer	Years BP
2	17	9640 ± 190 – 9100 ± 150
3	7	5610 ± 110
1	5	6150 ± 105[a]

N: number of coprolite analyzed per layer to test the accuracy of the classification function.
[a] There is a problematic dating from charcoal of 3080 ± 70 years BP that has been excluded.

3.3. Morphological analysis of modern faeces

Pellets obtained from each faecal sample were photographed. Multiple predictor variables such as length, wide, volume, perimeter, as well as the circularity and the length/wide relation, as measures of shape of faeces, were calculated by the Image J (Java 1.6.0_12) Program. Pellet thickness was also calculated with a digital caliper.

A Principal Component Analysis (PCA) using the computer program PCO (Anderson, 2003) was performed in order to allow the observation of most salient data pattern and structure (Legendre and Legendre, 1998; Anderson, 2003). Once the dispersion of the recent and archaeological dung data was observed, a Generalized Discriminant Analysis based on Euclidean distance was applied. With this analysis it could be determined if the predictor variables are useful to differentiate faeces of guanaco and huemul. A discriminant classification function, based on the reference collection, could be used to identify coprolites of unknown origin. The number of principal component axes was chosen as the number of axes resulting in the minimum mis-classification error by the leave-one out method (Lachenbruch and Mickey, 1968; Anderson and Willis, 2003; Anderson and Robinson, 2003). It was additionally tested if there were significant differences between groups of recent faeces by using the trace statistics and obtaining a *p*-value by permutation (Anderson, 2001). Statistical analyses were performed with the computer program CAP (Anderson, 2004). It was also determined which of the predictor variables contribute more in the discrimination, through the Pearson Correlation Index.

The accuracy of the classification function was estimated as the number of examined faeces that were correctly reassigned to their

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zoological origin by leave-one-out procedure (Lachenbruch and Mickey, 1968). Furthermore, new observations corresponding to recent faecal pellets of guanaco ($n = 4$) and huemul ($n = 5$) collected from the same studied areas, were also classified by their pellet dimensions to test the accuracy of the classification function.

3.4. Accuracy of the classification function for coprolites identification

Camelid's coprolites ($n_{pellets} = 6$) were used to test the accuracy of the discriminant classification function of modern faeces as references to identify coprolites.

4. Results

The first two principal component axes explained 99.8% of the total data variation, indicating that the ordination is highly likely to have captured the majority of the salient patterns in the multidimensional data cloud. As shown in Fig. 1, modern faecal pellets of guanaco and huemul lie in two separate groups using the PCA. Coprolites were grouped within the guanaco pellets group (Fig. 1).

Pellet size and shape were able to discriminate faeces of guanaco from those of huemul (Pillai's trace = 0.93, $p = 0.0001$ using 9999 permutations). The variable that most contributed to the discrimination of faecal groups was the volume, as it was the variable with the highest rate of correlation with the first discriminant axis (Pearson = 0.95). The accuracy of the classification function for modern faeces was 100%, as all the faeces of guanaco and huemul used in the previous analysis were correctly classified. Similarly, all the faeces of known origin collected from the studied area were correctly classified by the discriminant classification function. From the 6 coprolites assigned to camelids by paleoparasitological analyses, all were correctly classified as camelids, grouped within the guanaco pellets assemblage.

5. Discussion

During recent decades, studies of coprolites have increased all over the world, although not much attention has been focused on the zoological origin of archaeological dung. Identification of coprolites implies the observation of their macroscopic features, such as shape, size, weight, odor, and color of the rehydrated solution (Callen and Cameron, 1960; Reinhard and Bryant, 1992, 2008; Chame, 2003; Bryant and Dean, 2006; Linseele et al., 2010). Features like odor and color are biased through the observer, so reliable identification is difficult to achieve (Reinhard and Bryant, 1992). Erroneous identifications can increase when distinguishing among coprolites of related animals. The examination of coprolites contents can be often relevant to identify the zoological origin (Reinhard, 1992; Linseele et al., 2010, 2013), i.e., the finding of specific endoparasites.

Accurate identification of coprolites could also be obtained by the molecular identification of the ancient DNA (aDNA). However, its potential use depends on the preservation of the aDNA, which is not always recovered (Hoss et al., 1996; Hofreiter et al., 2001).

In the present study, the utilization of the discriminant analysis function by the combination of both pellet shape and size measurements, demonstrated its efficacy in the identification of ancient and modern large Patagonian artiodactyls pellets, particularly for guanaco and huemul.

The high accuracy (100%) of guanaco and huemul identification of recent faeces registered in the present study is relevant, as previous tests of the reliability of the identification of recent dung provided variable results (Farrell et al., 2000; Davison et al., 2002; Zuercher et al., 2003; Prugh and Ritland, 2005; Bowkett et al., 2009). Some of the studies obtained an accuracy around 80% (Farrell et al., 2000; Prugh and Ritland, 2005; Bowkett et al., 2009), and others concluded that results obtained by the measure of the pellet size alone was not able to identify faeces (Davison et al., 2002). However, those studies were limited to the identification of recent dung by field observation or by the utilization of simple models, using a few dimensions rather than the combination of multiple measurements from pellets, as was applied in the present research.

Recently, the used of statistical analysis of morphometric features based on a reference collection of recent dung, allowed the identification of archaeological dung as dung of small gazelles (Linseele et al., 2010; Riemer, 2011). However, another archaeological dung sample was not able to be distinguished by this analysis.

In the present study, coprolites collected from layer 17 dated 9640 ± 190 B.P. were also classified as guanaco. However, until early Holocene the camelids of Patagonia included the guanaco and two extinct camelids *Lama owenii* and *Lama gracilis*. Guanaco is the only extant camelid inhabiting Patagonia from 9000 BP (Miotti and Salemme, 1999). Thus, coprolites of early layers could correspond to those of guanaco or to some of the extinct taxa. However, in the paleontological and archaeological record of Patagonia guanacos greatly outnumber *L. gracilis*, and remains of *Lama oweni* were recorded only in the Magellan area (Miotti and Salemme, 1999). In addition, the fact that there was no evidence of the extinct camelids in the archaeological record of Cerro Casa de Piedra, cave 7, it is likely that coprolites of the early Holocene layers examined were also from guanaco.

The present is the first to perform a general discriminant analysis of modern faeces to identify coprolites. This is also the first research that takes into account the identification error. The use of this statistical tool along with paleoparasitological examination of coprolites allowed the estimation of archaeological dung identification error.

Although taphonomic processes due to the sediment pressure could alter the morphology of coprolites, in the present study this factor did not influence coprolite preservation, as coprolites collected from layer 17, dated 9640 ± 190 B.P., under more pressure than the upper layers, were also correctly classified as camelids. Similarly, Linseele et al. (2010) did not found variation in shape and size of archaeological dung compared with recent faeces. The high accuracy of the discriminant classification function of modern faeces to identify coprolites highlights its use as a reliable, cheap, and useful tool to distinguish artiodactyls coprolites collected from Patagonian archaeological sites.

6. Conclusion

This is the first study that used Generalized Discriminant Analysis of modern faeces as a tool to identify coprolites. This is also the first contribution to the morphometric identification of Patagonian artiodactyls dung. It was demonstrated that this is a reliable, cheap, non-invasive, and appropriate method that can produce accurate results for paleoecological studies. It has also provided the basis for its potential implementation to identify coprolites of other sympatric animals associated with archaeological sites.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.quaint.2013.12.055.

References

Anderson, M.J., 2001. Permutation tests for univariate or multivariate analysis of variance and regression. Canadian Journal of Fisheries and Aquatic Sciences 58, 626–639.

Anderson, M.J., 2003. PCO: a FORTRAN Computer Program for Principal Coordinate Analysis. Department of Statistics, University of Auckland, New Zealand.

Anderson, M.J., 2004. CAP: a FORTRAN Computer Program for Canonical Analysis of Principal Coordinates. Department of Statistics, University of Auckland, New Zealand.

Anderson, M.J., Robinson, J., 2003. Generalized discriminant analysis based on distances. Australian and New Zealand Journal of Statistics 45, 301–318.

Anderson, M.J., Willis, T.J., 2003. Canonical analysis of principal coordinates: a useful method of constrained ordination for ecology. Ecology 84, 511–525.

Aschero, C.A., 1996. El Área Río Belgrano-Lago Posadas (Santa Cruz): problemas y estado de problemas. In: Gómez Otero, J. (Ed.), Arqueología. Solo Patagonia. CENPAT-CONICET, Puerto Madryn, pp. 17–26.

Araújo, A.J.G., Ferreira, L.F., Camillo-Coura, L., Gonçalves, M.L.C., 2000. Parasitos, parasitismo e paleoparasitologia. Anais da Academia Nacional de Medicina. Rio de Janeiro 160, 20–27.

Bryant, V.M., Dean, G.W., 2006. Archaeological coprolite science: the legacy of Eric O. Callen (1912–1970). Palaeogeography, Palaeoclimatology, Palaeoecology 237, 51–66.

Bowkett, A.E., Plowman, A.B., Stevens, J.R., Tim, R.B., Davenport, T.R.B., Van Vuuren, B.J., 2009. Genetic testing of dung identification for antelope surveys in the Udzungwa Mountains, Tanzania. Conservation Genetics 10, 251–255.

Callen, E.O., Cameron, T.W.M., 1960. A prehistoric diet revealed in coprolites. New Scientist 8, 35–40.

Chame, M., 2003. Terrestrial mammal faeces: a morphometric summary and description. Memórias do Instituto Oswaldo Cruz 98, 71–94.

Chin, K., 2002. Analyses of coprolites produced by carnivorous vertebrates. In: Kowalewski, M., Kelley, P.H. (Eds.), Predation in the Fossil Record, Paleontology Society Special Papers, vol. 8, pp. 43–49.

Civalero, M.T., Aschero, C., 2003. Early occupations at Cerro Casa de Piedra 7, Santa Cruz Province, Patagonia, Argentina. In: Miotti, L., Salemme, M., Flegenheimer, N. (Eds.), Where the South Winds Blow. Texas A&M University, pp. 141–148.

Civalero, M.T., Franco, N.V., 2003. Early human occupations in Western Santa Cruz Province, Southernmost South America. Quaternary International 109-110, 77–86.

Davison, A., Birks, J.D.S., Brookes, R.C., Braithwaite, T.C., Messenger, J.E., 2002. On the origin of faeces: morphological *versus* molecular methods for surveying rare carnivores from their scats. Journal of Zoology 257, 141–143.

De Nigris, M.E., 2007. Nuevos datos, viejas colecciones: los conjuntos óseos de Cerro Casa de Piedra Cueva 5 (Parque Nacional Perito Moreno, Santa Cruz). Intersecciones en Antropología 8, 253–264.

Dittmar, K., Teegen, W.R., 2003. The presence of *Fasciola hepatica* (liver fluke) in human and cattle from a 4500 years old archaeological site in the Saale – Unstrut Valley, Germany. Memórias do Instituto Oswaldo Cruz 98, 141–144.

Farrell, L.E., Roman, J., Sunquist, M.E., 2000. Dietary separation of sympatric carnivores identified by molecular analysis of scats. Molecular Ecology 9, 1583–1590.

Hofreiter, M., Serre, D., Poinar, H.N., Kuch, M., Pääbo, S., 2001. Ancient DNA. Nature Reviews Genetics 2, 353–359.

Hoss, M., Jaruga, P., Zastawny, T.H., Dizdaroglu, M., Paabo, S., 1996. DNA damage and DNA sequence retrieval from ancient tissues. Nucleic Acids Research 24, 1304–1307.

Lachenbruch, P.A., Mickey, M.R., 1968. Estimation of error rates in discriminant analysis. Technometrics 10, 1–11.

Legendre, P., Legendre, L., 1998. Numerical Ecology, second English ed. Elsevier Science B.V., Amsterdam.

Linseele, V., Marinova, E., Van Neer, W., Vermeersch, P.M., 2010. Sites with Holocene dung deposits in the Eastern Desert of Egypt: visited by herders? Journal of Arid Environments 74, 818–828.

Linseele, V., Riemer, H., Baeten, J., De Vos, D., Marinova, E., Ottoni, C., 2013. Species identification of archaeological dung remains: A critical review of potential methods. Journal of Environmental Archaeology 18, 5–17.

Miotti, L., Salemme, M., 1999. Biodiversity, taxonomic richness and specialist-generalist during Late Pleistocene/Early Holocene times in Pampa and Patagonia (Argentina, southern South America). Quaternary International 53, 53–68.

Prugh, L.R., Ritland, C.E., 2005. Molecular testing of observer identification of carnivore faeces in the field. Wildlife Society Bulletin 33, 189–194.

Putman, R.J., 1984. Facts from faeces. Mammal Review 14, 79–97.

Reinhard, K.J., Bryant, M.V., 2008. Pathoecology and the future of coprolite studies in Bioarchaeology. In: Stodder, A.W.M. (Ed.), Reanalysis and Reinterpretation in Southwestern Bioarchaeology. Arizona State University Press, Tempe, pp. 205–224.

Reinhard, K.J., Bryant, V.M., 1992. Coprolite Analysis: A Biological Perspective on Archaeology. In: Natural Resources, School of Papers in Natural Resources. University of Nebraska, Lincoln, pp. 245–288.

Reinhard, K.J., Hamilton, D.L., Hevly, R.H., 1991. Use of pollen concentration in paleopharmacology: coprolite evidence of medicinal plants. Journal of Ethnobiology and Ethnomedicine 11, 117–132.

Reinhard, K.J., 1992. Parasitology as an interpretative tool in archaeology. American Antiquity 57, 231–245.

Riemer, H., 2011. Animal dung. In: Riemer, H. (Ed.), El Kharafish. The Archaeology of Sheikh Muftah Pastoral Nomads in the Desert Around Dakhla Oasis (Egypt) (Africa Praehistorica 25). Heinrich Barth Institute, Cologne, pp. 146–161.

Taglioretti, V., 2008. Estudio paleoparasitológico de coprolitos de camélidos procedentes del sitio arqueológico Cerro Casa de Piedra 7 (CCP7), Patagonia, Argentina (Licenciate thesis). Universidad Nacional de Mar del Plata, Argentina, p. 80.

Taglioretti, V., Fugassa, M.H., Sardella, N.H., 2009. Paleoparasitología de camélidos de Patagonia: nuevos datos. Acta Bioquímica Clínica Latinoamericana 1, 148.

Zuercher, G.L., Gipson, P.S., Stewart, G.C., 2003. Identification of carnivore faeces by local peoples and molecular analyses. Wildlife Society Bulletin 31, 961–970.

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