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Improved coprolite identification in Patagonian archaeological contexts

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ARTICLE INFO

Article history:

Received 19 December 2016
Received in revised form
2 March 2017
Accepted 9 March 2017
Available online xxx

Keywords:

Ancient DNA
Parasitology
Environmental archaeology
Puma concolor

ABSTRACT

In the present work, we report a molecular approach that contributes to determine the zoological origin of unidentified coprolites from Patagonia. Three coprolites with morphological characters attributable to human or carnivorous, from Patagonian archaeological sites, were analyzed. Molecular analysis was conducted following the Authenticity Criteria to Determine Ancient DNA (aDNA) sequences. Two replicates of 398 and 521 coprolites were amplified. Specific fragments of *cytb* and *nadh5* genes were successfully sequenced. No PCR positives were reached to any aDNA replicates of coprolite 246. In the present work mammal coprolites aDNA between 2,700–5,100 years old were isolated. These results allowed us to establish coprolites identity as *Puma concolor*. This is the first genomic information produced in an Argentinian lab based on aDNA from Patagonian coprolites. In consequence, the present study would open the way to future paleogenomics studies in archaeological sites of the region.

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1. Introduction

Coprolites are dehydrated or mineralized feces from ancient fauna. These ancient feces have been preserved over time. In Holocene archaeological contexts coprolites are dehydrated feces and they have mainly been found in caves, rock shelters and in open-air sites but with low frequency. Coprolites have been reported being part of caves or rock shelters floor but frequently there are other deposits associated to feces, as human latrines (e.g., Pike, 1968; Moore, 1981; Fernandes et al., 2005), rodent middens (Fugassa, 2014) and feces from mummified remains, denominated enteroliths. Palynological, botanical, zoological, entomological, molecular and biochemical studies have been performed to recover information about diet (e.g., Reinhard et al., 1992; 2007; Bon et al., 2012), sex (Sutton et al., 1996; Rhode, 2003), identification (Poinar et al., 2003), phylogeny (Bon et al., 2012), health and culture (e.g. Reinhard, 1988; Faulkner et al., 2000; Bouchet et al., 2003; Santoro et al., 2003) of its defecator.

In Argentina, coprolites have been analyzed in palynological (D'Antoni and Togo, 1974; Velázquez et al., 2010), botanical (Martínez and Yaguédú, 2012) and parasitological (e.g., Fugassa,

2006; Beltrame et al., 2010; Sardella and Fugassa, 2009; Taglioretti et al., 2015) studies. However, coprolite analysis based on ancient DNA (aDNA) has not yet been conducted in Argentina. Knowledge about coprolites zoological origins is critical to complement biological and cultural analysis obtained in Patagonian sites. Misdiagnosis could distort subsequent paleoecological and anthropological inferences.

Morphological identification of coprolites involves observation of macroscopic features, such as shape, size, weight, smell and color of the rehydrated solution (Chame, 2003; Bryant and Dean, 2006; Jouy-Avantin et al., 2003). However, characters as smell and color depend on the observer and shape, size and weight are subject to taphonomic processes that upset coprolites morphology. Diet content and specific parasites have contributed to the identification of the defecator animal (Reinhard, 1992; Linseele et al., 2010, 2013).

The identification of feces is a problem in both wildlife and in archaeology studies. Coprolites have been associated with hunter-gatherers in archaeological contexts of Patagonia. In archaeological sites of *Cerro Casa de Piedra* (Santa Cruz Province, Argentina) a large number of coprolites were found. In some cases, coprolites zoological origin could not be defined. The difficulty to distinguish between hunter-gatherer and carnivore coprolites hindered the correct interpretation of the obtained parasitological evidences. In this sense, molecular analysis of coprolites is a useful tool. This method is based on identification of DNA from sloughed colonic

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epithelial cells (Albaugh et al., 1992). Mitochondrial DNA contains informative regions to allow discriminate between mammalian carnivores (e.g. Farrell et al., 2000; Roques et al., 2011; Naidu et al., 2012). In the last years, genomic information has contributed to identification of current and ancient feces. Ancient DNA (aDNA) sequencing advances have produced new information that complement morphometric features (e.g. Rasmussen et al., 2010; Bon et al., 2012; Caragiulo et al., 2014; Metcalf et al., 2016). In the present work, we report a molecular approach that helps to define the zoological origin of unidentified coprolites from archaeological sites of Patagonia.

2. Materials and methods

Three coprolites with morphological characters attributable to human or carnivorous, from *Cerro Casa de Piedra*, were analyzed. *Cerro Casa de Piedra* is a volcanic hill located in the National Park Perito Moreno to the west of Santa Cruz province, Argentina. Coprolite 398 was extracted from layer II of cave 5 (CCP5), dated in $2,740 \pm 100$ years (yr) before present (B.P.). CCP5 site is a deep cave containing 7 levels and the human occupation takes places in three periods ca. 6,780–6,540 yr B.P., ca. 5,170–4,330 yr B.P. and ca. 2,740–2,550 yr B.P. (Aschero, 1996). Coprolite 398 (Fig. 1A) showed a dark brown color and a large number of light brown short hairs and it exhibited an intense fecal odor after rehydration. According to morphological characters and its maximum width of 49.3 mm, coprolite 398 was attributable to *Puma concolor*, *Panthera onca* or human because its diameter greater than *Pseudalopex culpaeus* feces, the highest canid in the area (Cornejo Farfán and Jiménez Milón, 2001). Coprolites 521 and 246 came from cave 7 (CCP7), layer IV dated in $5,120 \pm 90$ – $3,970 \pm 80$ yr B.P. and layer XVII/XVIII, dated in $9,100 \pm 150$ – $10,530 \pm 620$ yr B.P., respectively. CCP7 archaeological site displays a stratigraphic sequence of 19 levels showing human occupation between ca. $9,700 \pm 100$ and $3,600 \pm 70$ yr B.P. (Aschero, 1996; Civalero and Aschero, 2003). Coprolite 521 (Fig. 1B) had four extremely calcareous fragments with similar aspect to felids previously found in CCP5 site (Fugassa et al., 2009). Smell of rehydrated fragments was compatible with fox and felids but diameter was higher, 34.3 mm, exceeding usual diameter of fox coprolites. Coprolite 246 (Fig. 1C) was dark brown, no recognizable odor after rehydration was detected. There were no identified macroscopic organic remains. Morphology and diameter of 27.8 mm suggested that this coprolite could be attributed to human.

Molecular analysis were conducted following the Authenticity Criteria to Determine Ancient DNA Sequences (Hofreiter et al., 2001; Willerslev and Cooper, 2005; Fulton, 2012). Special reagents for ancient molecular analysis were used. aDNA extraction, PCR, electrophoresis; PCR products purification and aDNA sequencing were carried out in separate places. Four replicates of 200–300 mg of each coprolite were analyzed. aDNA extraction was performed by using GENE CLEAN FOR ANCIENT DNA™ KIT (MP Biomedicals). DeHybernation solution A (guanidine-based solution) and homogenization Matrix/Tubes were selected to lysis protocol following manufacturer instructions. aDNA was eluted with 50 µl of DNA-free elution solution. Finally, aDNA solutions were concentrated by Genomic DNA Clean & Concentrator™-10 (Zymo Research) in a final volume of 30 µl.

Molecular identification was first tested by PCR amplification using specific carnivores and felids primers to avoid misdiagnosis by contamination of aDNA with current human DNA from different sources reducing the risk of false positives.

In consequence, two fragments of mitochondrial aDNA, one of 146 bp of *cytochrome b* (*cytb*) gene from carnivores (among them humans) (Farrell et al., 2000) and other of 160 bp of *nicotinamide*

adenine dinucleotide dehydrogenase subunit 5 (*nadh5*) gene from felid species (primer pair NAD5C2-F; NAD5C2-R) (Roques et al., 2011) were selected to amplify aDNA from coprolites. PCR reactions were performed in a final volume of 12.5 µl containing 1 µl of 10-fold dilution of DNA sample, 200 µM of each dNTP (ThermoScientific), 0.4 µM of each primer and 1 units of AmpliTaq Gold® DNA Polymerase (Apply biosystems, Life Technologies) in 10X PCR Buffer II, 2 mM of MgCl₂ and 5% of DMSO (Sigma-Aldrich). The PCR conditions were as follows: an initial denaturation step (95 °C for 10 min), 40 cycles at 94 °C for 30 s (denaturation), 50 °C for 30 s (annealing), and 72 °C for 45s (extension), and 72 °C for 5 min (final extension). A negative control was included in all PCR experiments. Duplicates of the specific fragment were sequenced in forward and reverse sense and chromatograms were analyzed using BioEdit v7.2.0 (copyright © 1997–2013, Tom Hall, Ibis Biosciences). The consensus sequences obtained were compared with the GenBank sequences by using the BLASTN algorithm (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) of the National Center for Biotechnology Information (NCBI). Sequences were aligned using the multiple alignment tool: Clustal W2.0.9 (Larkin et al., 2007). Pairwise analysis was performed between sequences and identity percentage was determined by using LALIGN (Expasy Bioinformatics Research tools).

3. Results and discussion

Two replicates of 398 and 521 coprolites were amplified. Specific fragments of *cytb* and *nadh5* genes were successfully sequenced. No PCR positives were reached to any aDNA replicates of coprolite 246. This coprolite had been attributed to human according to morphometric characters, but *cytb* amplification could not be reached. However, assignment of this coprolite to human could not be ruled out, DNA could be damaged due to the age of the sample ($9,100 \pm 150$ – $10,530 \pm 620$ yr B.P.).

A consensus nucleotide sequence of 170 bp of *cytb* was obtained to coprolite 398. BLASTN analysis showed 98% of identity with Brazilian (Accession number: KC567527.1) and Northeast Argentinian (KC567478.1) sequences (Evaluate 2e-58). A 97% of identity (Evaluate 7e-68) with North American *Puma concolor cytb* sequence (AH014071.2). These results confirmed the zoological origin of coprolite complementing the morphometric study. On the other hand, BLASTN of 168 bp consensus of *cytb* sequence of coprolite 521 brought an identity of 99% (Evaluate 2e-66) with sequences from Brazil (KC567527.1) and Argentina (KC567478.1). A 98% (Evaluate 4e-70) with North American *Puma concolor* sequence (AH014071.2). Previous mitochondrial DNA analysis has separated pumas in three groups: North American, Central American and South American (Caragiulo et al., 2014).

Pairwise analysis of *cytb* sequences between 398 and 521 coprolites showed an identity of 99.4%. These coprolites were collected from different archaeological sites but both are just 400 m from each other. On the other hand, comparison of 398 and 521 sequences with *cytb* sequence from *Puma concolor* skin hairs from fur made by Selk'nam (Santa Cruz province, historical time) (Petrih and Fugassa, 2015) showed a 98.8% of identity with 398 coprolite sequence and 98.2% with coprolite 521 sequence. *Cytochrome b* gene evolution has been widely studied, it is relatively constant among large terrestrial mammals fragment (Irwin et al., 1991) and this agree with results of *cytb* sequences of different ages. Nevertheless, *cytb* gene is informative and useful for species identification because it displays higher interspecies variation even studying short fragments (Tobe et al., 2009). In this sense, sequences of this work showed an 87% of identity with *cytb* sequence of *Panthera onca* (KP202264.1).

In addition, *nadh5* sequence allows to confirm *cytb* results, a 159

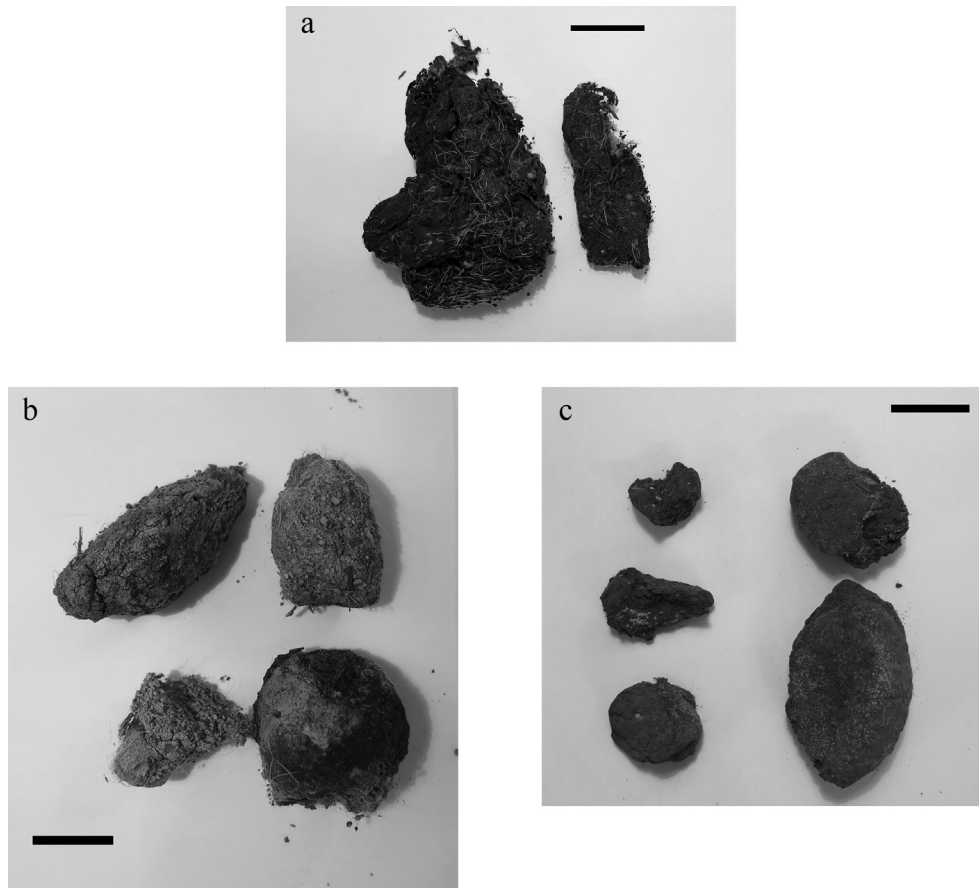


Fig. 1. Coprolites from Cerro Casa de Piedra archaeological site. A. Coprolite 398. B. Coprolite 521. C. Coprolite 246. Scale bar = 20 mm.

bp *nadh5* sequence supported coprolite identification with a 99% of identity (Evalue $5e-74$) with *Puma concolor* sequence (AH014071.2), and an 88% of identity with *nadh5* sequence (HM068603.1) from *Panthera onca*.

Pairwise analysis among *nadh5* sequences of coprolite 398 and GenBank sequences showed high identities between 98 and 99% with sequences from ancient (~11,000 yr B.P) *Puma concolor* (DQ857985 and KU884292) from Mylodon cave (Ultima Esperanza, Chile). Metcalf et al., 2016 reported that ancient Patagonian haplotypes of *nadh5* gene are closely related to haplotypes from current South American pumas.

In the present work aDNA from mammal coprolites between 2,700–5,100 years old was isolated, also *cytb* and *nadh5* fragment were amplified and sequenced in two coprolites samples indicating the good conservation of these coprolites over time.

Studies based on human coprolites identification have been performed in North America (Poinar et al., 2001; Gilbert et al., 2008). Poinar et al. (2001) have reported zoological origin and diet content (animal and vegetal) of 2,000 years old coprolites from Native Americans (Hinds Cave archaeological site, Texas, USA). American native haplogroups were identified by amplification of mitochondrial hypervariable region I and non-human animals and vegetal diet were defined by using mitochondrial and chloroplast genes, respectively. In the present work, the amplification of human aDNA was avoided because of risk of cross contamination with current human DNA. Also, coprolites were collected in the CCP sites prior to the widespread application of molecular techniques to ancient samples not following criteria to avoid contamination with current human DNA.

On the other hand, a downward movement of DNA through the layers under humidity and high temperature conditions has been postulated by some authors (Haile et al., 2007; Poinar et al., 2009). This DNA leaching could invalidate the identification of DNA from animals that inhabited in the corresponding stratigraphic layer. Poinar et al., 2009 suggested that DNA leaching should be considered as a source of contamination of human DNA from younger to older layers in Paisley Caves (Oregon, USA) archaeological sites. In a study performed in the same sites, leaching hypothesis was not accepted (Gilbert et al., 2009; Jenkins et al., 2013).

Given the extreme dry and cold conditions of the CCP5 and CCP7 sites and that there is no evidence of water leaks in the excavated area (Civalero and Aschero, 2003; De Nigris, 2004; Mancini, 2007), DNA leaching hypothesis for these sites is unlikely.

Nonetheless, morphological characters of 398 and 521 coprolites offered previous evidence of probable felid origin, later supported by molecular analysis. Both analyses provided an accurate zoological identification.

This work provides a useful strategy based on molecular tools that allow unbiased identification of coprolites. This approach complements macroscopic and microscopic analysis of coprolites. One aDNA study made with South American samples has been based on hairs isolated from coprolite of *Mylodon darwini* found in Mylodon cave (Pleistocene Chilean Patagonia). However, this ancient molecular study was carried out in Canada (Clack et al., 2012). Therefore, this is the first coprolite ancient genomic information from Patagonian Holocene performed in a regional laboratory. Molecular information could answer unresolved questions for another coprolites found in CCP5 and CCP7 archaeological sites.

Acknowledgements

We would like to thank to archaeologists Maria Teresa Civalero (INAPL, CONICET) and Carlos Aschero (UNT, CONICET) that conducted fieldwork providing the important collection of coprolites from CCP5 and CCP7. We also thank to ETC Internacional S.A. (Argentina) for providing us GENELEAN FOR ANCIENT DNA™ KIT (MP Biomedicals). Finally, we thank to Lic. José Marchisio for English editing. This study was funded by the National University of Mar del Plata (EXA680), CONICET (PIP090) and FonCyT (PICT 2316 and PICT3126).

References

- Albaugh, G.P., Iyengar, V., Lohani, A., Malayeri, M., Bala, S., Nair, P.P., 1992. Isolation of exfoliated colonic epithelial cells, a novel, non-invasive approach to the study of cellular markers. *Int. J. Cancer* 52 (3), 347–350.
- 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*. CONICET. Bs.As, Argentina.
- Bon, C., Berthonaud, V., Maksud, F., Labadie, K., Poulain, J., et al., 2012. Coprolites as a source of information on the genome and diet of the cave hyena. *Proc. R. Soc. Lond. B Biol. Sci.* 279 (1739), 2825–2830.
- Beltrame, M.O., Fugassa, M.H., Sardella, N.H., 2010. First paleoparasitological results from late Holocene in Patagonian coprolites. *J. Parasitol.* 96 (3), 648–651.
- Bouchet, F., Guidon, N., Dittmar, K., Harter, S., Ferreira, L.F., Chaves, S.M., Reinhard, K., Araujo, A., 2003. Parasite remains in archaeological sites. *Mem. Inst. Oswaldo Cruz* 98, 47–52.
- Bryant, V.M., Dean, G.W., 2006. Archaeological coprolite science: the legacy of Eric O. Callen (1912–1970). *Palaeogeogr. Palaeoclimatol. Palaeoecol.* 237 (1), 51–66.
- Caragiulo, A., Dias-Freedman, I., Clark, J.A., Rabinowitz, S., Amato, G., 2014. Mitochondrial DNA sequence variation and phylogeography of Neotropical pumas (*Puma concolor*). *Mitochondrial DNA* 25 (4), 304–312.
- Chame, M., 2003. Terrestrial mammal feces: a morphometric summary and description. *Mem. Inst. Oswaldo Cruz* 98, 71–94.
- Civalero, M.T., Aschero, C., 2003. Early occupations at Cerro Casa de Piedra 7, Santa Cruz Province, Patagonia, Argentina. Center for the Study of the First Americans. Texas A&M University Press, College Station, Texas, pp. 141–147.
- Clack, A.A., MacPhee, R.D., Poinar, H.N., 2012. *Mytilodon darwini* DNA sequences from ancient fecal hair shafts. *Ann. Anat. Anat. Anz.* 194 (1), 26–30.
- Cornejo Farfán, A., Jiménez Milón, P., 2001. Dieta del zorro andino *Pseudalopex culpaeus* (Canidae) en el matorral desértico del sur de Perú. *Rev. Ecol. Latinoam.* 8, 1–9.
- D'Antoni, H., Togo, J., 1974. Análisis polínico de coprolitos animales: su aplicación en arqueología. In: *Actas III Congreso Argentino de Arqueología*, Argentina, pp. 1–16.
- De Nigris, M.E., 2004. El consumo en grupos cazadores recolectores. Un ejemplo zooarqueológico de Patagonia Meridional. Ph.D. Thesis. Sociedad Argentina de Antropología, Buenos Aires, Argentina, p. 242.
- Farrell, L.E., Roman, J., Sunquist, M.E., 2000. Dietary separation of sympatric carnivores identified by molecular analysis of scats. *Mol. Ecol.* 9 (10), 1583–1590.
- Faulkner, C.T., Cowie, S.E., Martin, P.E., Martin, S.R., Shane Mayes, C., Patton, S., 2000. Archeological evidence of parasitic infection from the 19th century company town of Fayette, Michigan. *J. Parasitol.* 86 (4), 846–849.
- Fernandes, A., Ferreira, L.F., Gonçalves, M.L.C., Bouchet, F., Klein, C.H., et al., 2005. Intestinal parasite analysis in organic sediments collected from a 16th-century Belgian archeological site. *Cad. Saúde Públ.* 21 (1), 329–332.
- Fugassa, M.H., 2006. Enteroparasitosis en Poblaciones Cazadoras-Recolectoras de Patagonia Austral. Ph.D. Thesis. Universidad Nacional de Mar del Plata, Argentina, p. 274.
- Fugassa, M.H., Beltrame, M.O., Bayer, M.S., Sardella, N.H., 2009. Zoonotic parasites associated with felines from the Patagonian Holocene. *Mem. Inst. Oswaldo Cruz* 104 (8), 1177–1180.
- Fugassa, M.H., 2014. Registros parasitológicos en *rodent middens* del Parque Nacional Perito Moreno, Santa Cruz, Argentina. *Rev. Argent. Parasitol.* 3 (1), 6–11.
- Fulton, T.L., 2012. Setting up an ancient DNA laboratory. *Anc. DNA Methods Protoc.* 1–11.
- Gilbert, M.T.P., Jenkins, D.L., Götherstrom, A., Naveran, N., Sanchez, J.J., Hofreiter, M., et al., 2008. DNA from pre-Clovis human coprolites in Oregon, North America. *Science* 320 (5877), 786–789.
- Gilbert, M.T.P., Jenkins, D.L., Higham, T.F., Rasmussen, M., Malmstrom, H., Svensson, E.M., et al., 2009. Response to Comment by Poinar, et al. on "DNA from Pre-Clovis Human Coprolites in Oregon, North America". *Science* 325 (5937), 148.
- Haile, J., Holdaway, R., Oliver, K., Bunce, M., Gilbert, M.T.P., Nielsen, R., et al., 2007. Ancient DNA chronology within sediment deposits: are paleobiological reconstructions possible and is DNA leaching a factor? *Mol. Biol. Evol.* 24 (4), 982–989.
- Hofreiter, M., Serre, D., Poinar, H.N., Kuch, M., Pääbo, S., 2001. Ancient DNA. *Nat. Rev. Genet.* 2 (5), 353–359.
- Irwin, D.M., Kocher, T.D., Wilson, A.C., 1991. Evolution of the cytochrome-*b* gene of mammals. *J. Mol. Evol.* 32, 128–144.
- Jenkins, D.L., Davis, L.G., Stafford Jr., T.W., Campos, P.F., Connolly, T.J., Cummings, L.S., et al., 2013. Geochronology, archaeological context, and DNA at the Paisley caves. *Paleoamerican Odyssey* 32, 485–510.
- Jouy-Avantin, F., Debenath, A., Moigne, A.M., Moné, H., 2003. A standardized method for the description and the study of coprolites. *J. Archaeol. Sci.* 30 (3), 367–372.
- Larkin, M.A., Blackshields, G., Brown, N.P., Chenna, R., et al., 2007. Clustal W and Clustal X version 2.0. *Bioinformatics* 23 (21), 2947–2948.
- 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? *J. Arid Environ.* 74 (7), 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. *Environ. Archaeol.* 18 (1), 5–17.
- Mancini, M.V., 2007. Cambios paleoambientales en el ecotono bosque-estepa: análisis polínico del Sitio Cerro Casa de Piedra 7, Santa Cruz (Argentina). In: *Actas de la 6ta Jornada de Arqueología de Patagonia*, Punta Arenas, Chile, pp. 89–93.
- Martínez, A.C., Yaguéddú, C., 2012. Identificación de microrrestos vegetales en un coprolito humano del sitio Cerro Casa de Piedra, Santa Cruz, Argentina. *Magallania (Punta Arenas)* 40 (1), 333–339.
- Metcalf, J.L., Turney, C., Barnett, R., Martin, F., Bray, S.C., Vilstrup, J.T., et al., 2016. Synergistic roles of climate warming and human occupation in Patagonian megafaunal extinctions during the Last Deglaciation. *Sci. Adv.* 2 (6), e1501682.
- Moore, P.D., 1981. Life seen from a medieval latrine. *Nature* 294, 614.
- Naidu, A., Fitak, R.R., Munguia-Vega, A., Culver, M., 2012. Novel primers for complete mitochondrial cytochrome b gene sequencing in mammals. *Mol. Ecol. Resour.* 12 (2), 191–196.
- Petrih, R., Fugassa, M., 2015. Información molecular obtenida a partir de pieles de la colección del Museo Regional Fagnano, Río Grande, Tierra del Fuego/Molecular data from furs belonging to the Fagnano Regional Museum collection, Río Grande, Tierra del Fuego. *Rev. Argent. Antropol. Biol.* 17 (1), 28–34.
- Pike, A.W., 1968. Recovery of helminth eggs from archaeological excavations, and their possible usefulness in providing evidence for the purpose of an occupation. *Nature* 219, 303–304.
- Poinar, H.N., Kuch, M., Sobolik, K.D., Barnes, I., Stankiewicz, A.B., Kuder, T., et al., 2001. A molecular analysis of dietary diversity for three archaic Native Americans. *Proc. Natl. Acad. Sci.* 98 (8), 4317–4322.
- Poinar, H., Kuch, M., McDonald, G., Martin, P., Pääbo, S., 2003. Nuclear gene sequences from a late Pleistocene sloth coprolite. *Curr. Biol.* 13 (13), 1150–1152.
- Poinar, H., Fiedel, S., King, C.E., Devault, A.M., Bos, K., Kuch, M., Debruyne, R., 2009. Comment on "DNA from pre-Clovis human coprolites in Oregon, North America". *Science* 325 (5937), 148–148.
- Rasmussen, M., Li, Y., Lindgreen, S., Pedersen, J.S., Albrechtsen, A., et al., 2010. Ancient human genome sequence of an extinct Palaeo-Eskimo. *Nature* 463 (7282), 757–762.
- Reinhard, K.J., 1988. Cultural ecology of prehistoric parasitism on the Colorado Plateau as evidenced by coprology. *Am. J. Phys. Anthropol.* 77 (3), 355–366.
- Reinhard, K.J., 1992. Parasitology as an interpretive tool in archaeology. *Am. Antiq.* 231–245.
- Reinhard, K.J., Geib, P.R., Callahan, M.M., Hevly, R.H., 1992. Discovery of colon contents in a skeletonized burial: soil sampling for dietary remains. *J. Archaeol. Sci.* 19 (6), 697–705.
- Reinhard, K.J., Bryant, V.M., Vinton, S.D., 2007. Comment on reinterpreting the pollen data from Dos Cabezas. *Int. J. Osteoarchaeol.* 17 (5), 531–541.
- Rhode, D., 2003. Coprolites from Hidden Cave, revisited: evidence for site occupation history, diet and sex of occupants. *J. Archaeol. Sci.* 30, 909–912.
- Roques, S., Adrados, B., Chavez, C., Keller, C., Magnusson, W.E., Palomares, F., Godoy, J.A., 2011. Identification of Neotropical felid faeces using RCP-PCR. *Mol. Ecol. Resour.* 11 (1), 171–175.
- Santor, C., Vinton, S.D., Reinhard, K.J., 2003. Inca expansion and parasitism in the Lluta Valley: preliminary data. *Mem. Inst. Oswaldo Cruz* 98, 161–163.
- Sardella, N.H., Fugassa, M.H., 2009. Paleoparasitological analysis of rodent coprolites in holocene samples from Patagonia, Argentina. *J. Parasitol.* 95 (3), 646–651.
- Sutton, M., Malik, M., Ogram, A., 1996. Experiments on the determination of gender from coprolites by DNA analysis. *J. Archaeol. Sci.* 23 (2), 263–267.
- Taglioretti, V., Fugassa, M.H., Sardella, N.H., 2015. Parasitic diversity found in coprolites of camelids during the Holocene. *Parasitol. Res.* 114 (7), 2459–2464.
- Tobe, S.S., Kitchener, A., Linacre, A., 2009. *Cytochrome b* or *cytochrome c oxidase subunit I* for mammalian species identification an answer to the debate. *Forensic Sci. Int.* 2, 306–307.
- Velázquez, N.J., Burry, L.S., Mancini, M.V., Fugassa, M.H., 2010. Coprolitos de camélidos del Holoceno como indicadores paleoambientales. *Magallania (Punta Arenas)* 38 (2), 213–229.
- Willerslev, E., Cooper, A., 2005. Review paper. Ancient DNA. *Proc. R. Soc. Lond. B Biol. Sci.* 272 (1558), 3–16.