Forensic Science International: Genetics 45 (2020) 102226

Contents lists available at ScienceDirect

Forensic Science International: Genetics

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

Case report

Utility of genetic variation in coat color genes to distinguish wild, domestic and hybrid South American camelids for forensic and judicial applications

Benito A. González[a,b], Ana María Agapito[c], Francisco Novoa-Muñoz[d], Juliana Vianna[e], Warren E. Johnson[f,g], Juan Carlos Marín[c,*]

[a] Laboratorio de Ecología de Vida Silvestre, Facultad de Ciencias Forestales y de la Conservación de la Naturaleza, Universidad de Chile, Chile
[b] South American Camelid Specialist Group, Species Survival Commission, International Union for Conservation of Nature
[c] Laboratorio de Genómica y Biodiversidad, Departamento de Ciencias Básicas, Facultad de Ciencias, Universidad del Bío-Bío, Chillán, Chile
[d] Departamento de Estadística, Facultad de Ciencias, Universidad del Bío-Bío, Concepción, Chile
[e] Departamento de Ecosistemas y Medio Ambiente, Facultad de Agronomía e Ingeniería Forestal, Pontificia Universidad Católica de Chile, Santiago, Chile
[f] Smithsonian Conservation Biology Institute, Smithsonian Institution, Washington DC, United States
[g] Walter Reed Biosystematics Unit, Smithsonian Institution, Suitland, MD, United States

ARTICLE INFO

Keywords:
Guanaco
Llama
Hybrid
Coat color genes
Genetic forensics

ABSTRACT

A molecular genetic protocol for distinguishing pure and hybrid South American camelids was developed to provide strong, quantifiable, and unbiased species identification. We detail the application of the approach in the context of a criminal case in the Andes Mountains of central Chile where the defendants were alleged to have illegally hunted three wild guanacos (*Lama guanicoe*), as opposed to hybrid domestic llama (*Lama glama*)/wild guanaco crosses, which are unregulated. We describe a workflow that differentiates among wild, domestic and hybrid South American camelids (*Lama* versus *Vicugna*) based on mitochondrial cytochrome b genetic variation (to distinguish between *Lama* and *Vicugna*), and *MC1R* and exon 4 variation of the *ASIP* gene (to differentiate wild from domestic species). Additionally, we infer the population origin and sex of each of the three individuals from a panel of 15 autosomal microsatellite loci and the presence or absence of the SRY gene. Our analyses strongly supported the inference that the confiscated carcasses corresponded with 2 male and 1 female guanacos that were hunted illegally. Statistical power analyses suggested that there was an extremely low probability of misidentifying domestic camelids as wild camelids (an estimated 0 % Type I error rate), or using more conservative approached a 1.17 % chance of misidentification of wild species as domestic camelids (Type II error). Our case report and methodological and analytical protocols demonstrate the power of genetic variation in coat color genes to identify hybrids between wild and domestic camelid species and highlight the utility of the approach to help combat illegal wildlife hunting and trafficking.

1. Introduction

Poaching and illegal trade of wildlife is recognized to be a major threat to biodiversity, affecting efforts to protect and recover populations of animals from overexploitation. During the last decade, a resurgence of illegal hunting on a global scale is endangering emblematic species in all terrestrial and marine ecosystems [1,2]. To counter these threats, the application of forensic genetic approaches has become an increasingly common tool for monitoring and investigating incidents of poaching and illegal trade [3–5], providing more precise and replicable tools to identify the species, population of origin, individual identity and relatedness of a variety of sample types [6–9]. South American camelids (SAC), both wild and domestic, often coexist temporally and spatially [10,11]. Wild and domestic species readily interbreed in the wild and in captive and managed settings and produce fertile hybrids [12]. This has complicated the use of traditional methods of molecular analyses for differentiating taxa beyond genus (e.g. using mitochondrial DNA and microsatellite markers) since at the phylogenetic level two reciprocal groups are obtained: the vicuña, *Vicugna vicugna*, - alpaca, *V. pacos* haplogroup and the guanaco, *Lama guanicoe* - llama, *L. glama* haplogroup [13].

The guanaco, classified as a Least Concern by the IUCN Red List [14], is protected nationally throughout its distribution. Permits are required for local use and for the international trade of derived products (since it is classified in the Appendix II of CITES). It is illegal to hunt guanacos without authorization in Chile and Argentina [15] and

* Corresponding author.
E-mail address: jcmarin@ubiobio.cl (J.C. Marín).

https://doi.org/10.1016/j.fsigen.2019.102226
Received 14 August 2019; Received in revised form 11 December 2019; Accepted 11 December 2019
Available online 12 December 2019
1872-4973/ © 2019 Elsevier B.V. All rights reserved.

commercialization (sale) of meat products within these countries and the European Union, the main international destination of legal meat products of non-domestic species, is regulated by resolutions and norms that are more stringent than those covering the production and sale of beef from domestic cattle (i.e. CITES permits and certificates Conf. 12.3 Rev. CoP17, Non-detrimental Findings report [16]). However, somewhat ironically, the sanctions for illegal wildlife poaching in Chile (Law N° 19.473) are less punitive than those for cattle rustling (Article N° 448, Chilean Penal Code). Complicating matters, the legal framework for protecting hybrid individuals in Chile is unclear, and there are no explicit protections beyond the interpretation of local norms and/or an extrapolation or interpretation of CITES regulations (Conf. 10.17 Rev. CoP14). This lack of a clear legal framework covering the exploitation and commerce of South American camelids has likely contributed to the perception and expectation that the harvest (hunting) of "hybrid species" might be significantly different than if they were pure wild, and would be treated less harshly by governing authorities.

Here we describe a workflow that applies a number of novel approaches and a logical hierarchical testing regime to accurately identify SAC. Additionally, we describe the successful resolution of a forensic case involving guanaco poaching, where the accused claimed to have hunted and slaughtered guanaco-llama hybrids (which would have been legal under current laws). We describe molecular genetic analyses that were used to identify with a high degree of certainty whether the three South American camelids were pure wild species (guanaco or vicuña) or domestic species (llama or alpaca) using genetic variants of the gene that codifies fur color in mammals and that was under strong selection during the domestication process [17]. Importantly, it was also possible to identify the population of origin (location) and the number of individuals involved (hunted) based on microsatellite genotyping. The sex of each individual was ascertained using specific Y-chromosome markers. Here, our analyses demonstrate that sequence variation in coat color genes in South American camelids is sufficient to distinguish pure wild species from hybrid and / or domestic species. We then provide an example of how our approach /case study, demonstrating the utility of our approach in helping resolve a complex case of illegal hunting.

2. Materials and methods

2.1. SAC identification method

To collect baseline data, we analyzed data from 79 guanacos and 89 vicuñas from throughout their distribution in Peru, Argentina and Chile, representing the subspecies of wild guanaco and wild vicuña, *L. g. cacsilencis/L. g. guanicoe* and *V. v. mensalis/V. v. vicugna* respectively. Similarly, we analysed 89 samples of domestic llamas, and 84 alpacas from Andean countries (Supplementary Table 1, Supplementary Information). Total genomic DNA was extracted using the most appropriate methods for each type of tissue. PCR amplification of the 5' end of the mitochondrial control region [18] was used to distinguish South American Camelids from other Artiodactyls (Fig. 1, step 1). Sequences were aligned using Geneious v.9.1.5 (Biomatters, Auckland, New Zealand) and were compared to sequences available on the NCBI database. Statistical confidence of sequence similarity within and among species was further assessed using BLAST [19]. Next, the genus of individuals was confirmed as either, *Lama* or *Vicugna* based on diagnostic melanocortin 1 receptor (*MC1R*) sequence variation (Fig. 1, step 2). Then, to determine if the confiscated samples corresponded to a wild or a domestic camelid, two biparental genes responsible for coat color in mammals were assessed (Fig. 1, step 3) using PCR primers designed to amplify the *MC1R* coding region [20] and portions of the *ASIP* coding and intronic regions [21]. In this region a specific set of 14 substitutions unequivocally differentiate wild from domestic South American camelids [17] (**F**igure 1, step 3, Supplementary Table 2A, 2B, 2C, 2D, Supplementary Information). These Single Nucleotide Polymorphisms (SNPs) were identified by sequence alignment (www.geneious.com) and were confirmed by resequencing the whole fragment in the opposite direction. Aligned sequence data for each gene were imported individually into DNASP 5.0 software [22] to analyze haplotype diversity and nucleotide diversity. The gametic phase of each haplotype was determined with the software BEAGLE Version 3.3.1 [23].

Samples were linked to specific individuals based on patterns of variation in a panel of 15 highly polymorphic microsatellites, also known as STRs or simple-tandem repeats, designed specifically for South American camelids [6] (Fig. 1, step 4). Fifteen autosomal dinucleotide microsatellite loci, designated YWLL08, YWLL29, YWLL36, YWLL38, YWLL40, YWLL43, YWLL44, YWLL46 [24], LCA5, LCA19, LCA22, LCA23 [25], LCA65 [26]) and LGU49, LGU68 [27] were amplified. The amplification was carried out as described in Marín et al. 2013 [18] and fragments analyzed on an ABI-3100 sequencer (Perkin Elmer Applied Biosystems). Data collection, sizing of bands and analyses were carried out using Genemarker v. 1.70 (SoftGenetics). Match probabilities between samples were estimated using Microsat ToolKit [28] without adjusting for sample size (given the size of the sample sets). Sex-specific amplification of the SRY gene was performed using the camel-specific primers SryB5 and B3 [6] to determine the sex of each sample (Fig. 1, step 5). The SRY gene ($\approx$175 bp) was amplified as in Marín et al. [6].

2.2. Assignment power

K-fold cross-validation and a Bayesian model were used to calculate Type I and II error in sample assignation. To validate our method and assess the impact of subsampling, we performed a K-fold cross-validation [29] using the sample function of the R program [30] to generate random numbers. For this exercise we divided the sample data into two 50 % groups [31]: one as a training set and the other as the test group (K = 2), without differentiating if they corresponded to guanacos, llamas, vicuñas or alpacas, and keeping half of samples as a 'hold-out' set, which did not contribute to the allele frequency estimates of the reference groups. This hold-out set served as the test control sample which was used to estimate assignment error. We repeated the procedure four times over 341 samples of camelids with known phenotypic and genetic information (Supplementary Table 2, Supplementary Information) and calculated average percent Type I and Type II error rates. Additionally, a commonly recommended Bayesian approach [3,32] was also generated using the same database. Samples of our case study were excluded for both statistical procedures.

2.3. Case history

Two people were arrested for transporting finely-butchered carcasses (for human consumption) of three unidentified South American camelids. These carcasses were discovered hidden in a 4 × 4 truck during a search of the vehicle as it was leaving a private ranch (~33°55′S, ~70°11′W). Carabineros de Chile (the Chile police force) and the Agricultural and Livestock Service administrative authorities were informed. These authorities confiscated the material and took tissue samples of the meat for genetic analyses. Due to the proximity of a well-monitored population of guanacos (*Lama guanicoe*) from the Laguna del Diamante Provincial Reserve in Argentina [33], it was immediately suspected that the confiscated specimens might be from these federally protected guanacos (*Lama guanicoe*). The suspects were charged with infringing the Hunting Law 19,473 for illegal possession of the products of a protected species (Case RUC 1500012957-4 in Prosecutor of Puente Alto, Santiago). During the hearing, the accused did not deny that the carcasses were "camelids". However, they alleged that the specimens they were carrying corresponded to guanaco/llama hybrids, or "llamanacos" (which are not protected by law) and they pointed out that approximately 20 km from where they were arrested

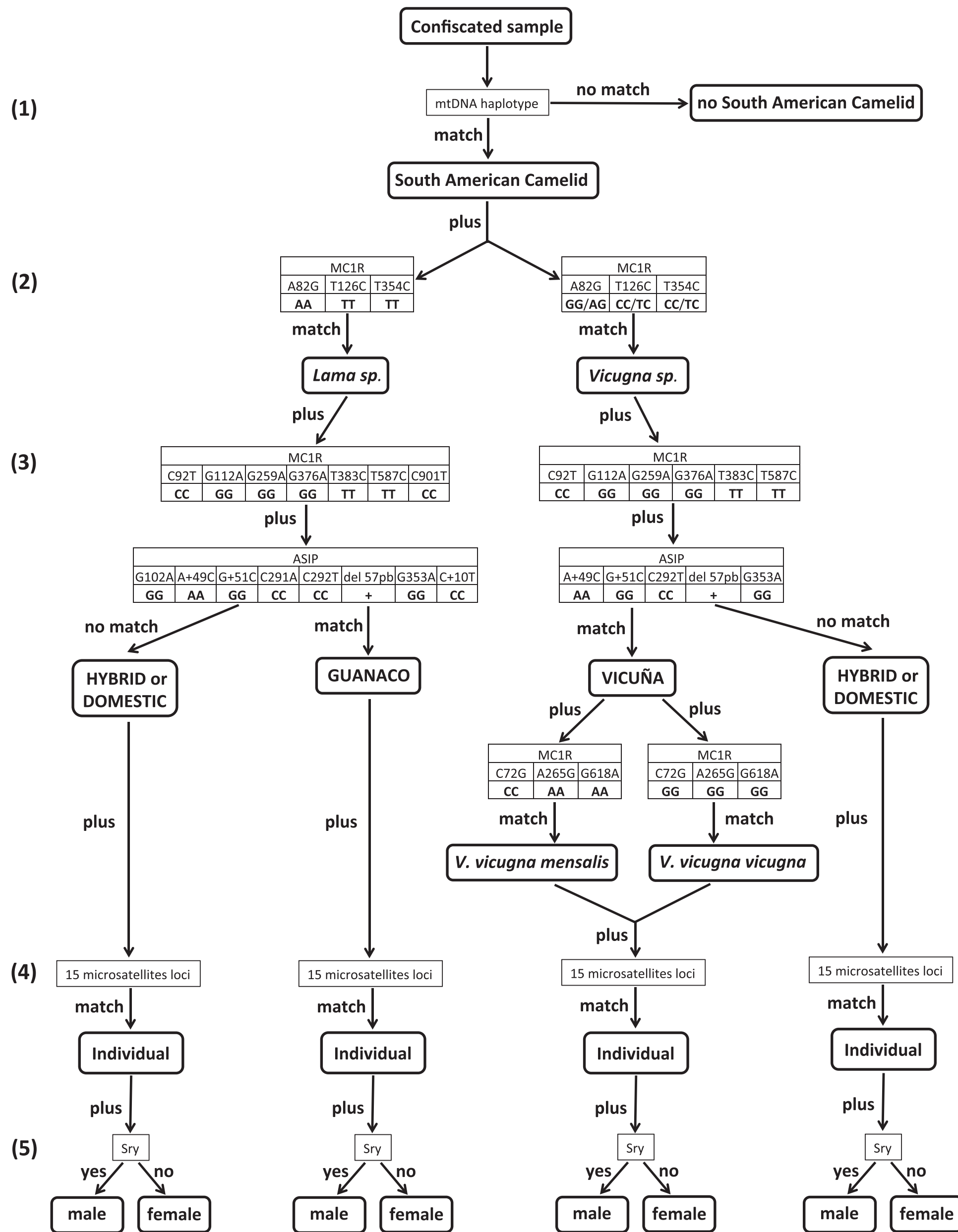

Fig. 1. Diagram of the five-step workflow through which individuals were classified by: (1) South American Camelid origin, (2) genus, (3) wild or domestic, (4) number of individuals, and (5) by sex (male or female). Classification of MC1R and ASIP haplotypes required exact matches. The haplotypes indicated are always homozygous except for Vicugna sp.'s MC1R haplotype.

there are numerous llama that could have interbred with wild guanacos.

From the evidence collected during the arrest, three muscle samples that were suspected to be from three different animals labeled as 1B2, 2B2 and 3B2, were sent to the Laboratorio de Genómica y Biodiversidad, Departamento de Ciencias Básicas, Universidad del Bío-Bío. Genomic DNA was extracted using standard proteinase K digestion and phenol/chloroform extraction procedures [34]. The hierarchical workflow described above was followed to resolve questions about the origins of the confiscated samples (Fig. 1).

3. Results and discussion

3.1. Estimation of assignment error

Our estimate of the error rate for distinguishing wild species from domestic species using our methodology was extremely low (calculated as 0 %). Similarly, the average estimated rate for misclassifying a domestic/hybrid as a wild individual was 0.58 % (using the K-fold cross-validation method), the second approach error estimates are considered 1.17 (Bayesian analysis) that is conservative with respect to the prosecution K-fold cross-validation method, but both error estimates are considered relevant, then in casework; indicating that the probability that an innocent person would be mistakenly determined to be guilty was only very marginally more likely (Supplementary Table 3, Supplementary Information). These analyses suggest that our approach has a probability (confidence rate) of over ∼99 % of being able to correctly distinguish domestic South American camelids from their wild progenitors. The Bayesian approach had a slightly higher estimated level Type II error (Supplementary Table 4, Supplementary Information) than the K-fold cross-validation method. These very low estimated error rates likely reflect historic low levels of admixture among species in this group [35]. Here, as is generally the case with most tests, the estimated frequency or probability of false positive was

of highest concern, especially since in prosecuting criminal cases a false positive would incorrectly determine that the sample was from a domestic animal and not a wild one (and in this case would be evidence of innocence). Although our methods are very robust, they also reinforce the general dictum that criminal cases should be resolved using a variety of independent types of evidence.

3.2. Mitochondrial DNA haplogroups

We obtained 300 base pairs of the 5′ end domain of the mitochondrial control region from the three confiscated meat samples. The sequences were aligned, and genetic sequence matched with South American Camelids haplotypes, rather than those of other Artiodactyls (Supplementary Table 5A, Supplementary Information).

3.3. Wild origin of samples of case

MC1R and Exon 4 of *ASIP* sequences revealed that samples 1B2, 2B2 and 3B2 had none of the 5 diagnostic mutations that characterize llamas and alpacas [17]. These mutations have never been observed in guanacos and are always in llamas [17] (Supplementary Table 5B, 5C, Supplementary Information). If these samples had been from a hybrid individual of first-generation guanaco and llama, these mutations would be detectable in at least one of the two chromosomal sets of the animal (a situation not observed here). Genetic variation in the MC1R sequences provided strong evidence the samples were either *Lama* (guanaco and llama) or *Vicugna* (vicuña and alpaca).

3.4. Individualization and sex identification of case samples

Results of the analysis of the microsatellite markers confirmed that the samples had to have come from three individual guanacos (Supplementary Table 6, Supplementary Information). Samples 1B2 and 3B2 were identified as males and sample 2B2 as a female.

4. Conclusions

Our research workflow, starting from a broad species-level scale to individual differentiation and sex determination, along with corresponding estimates of error rate to provide a robust measure of confidence in sample assignation, proved to be effective in determining the providence of samples of unknown species, including evidence that is useful for resolving cases in wildlife crimes. Our approach will be of special utility when the genetic purity (potential hybridization) is in question [36,37], as when domestic/hybrid products need to be distinguished from wild ones. The rigor and repeatability of the assays will be especially important when utilized in a legal context, as when evidence is introduced in the prosecution of criminal cases. These methodologies will also be useful to address other questions of genetic heritage, including the assessment of archaeological remains.

Here, we described a legal case where we used specific gene variation associated with coat color to differentiate wild and domestic animals in a case where traditional genetic analysis, i.e., mitochondrial or microsatellite markers [13,18,35] would not have been able to provide evidence of illegal hunting of a protected species. Patterns of sequence variation in the *MC1R* gene and exon 4 of the *ASIP* gene distinguished wild from domestic species with high confidence, helping to clarify that the three carcasses were from guanacos and that they were not hybrids, as was claimed. However, although our estimated error rates were minimal, genetic evidence perhaps should not be the only type of evidence to be used in a trial of these characteristics and independent types of corroborating evidence would clearly be of value.

We suspect that the guanaco carcasses would have been sold on the local black market where fresh or dried meat is in high demand during certain times of the year [38]. Although this species is protected by the Chilean Hunting Law, this legal instrument does not formally or explicitly address the legality of transactions between hunters (the sellers) and buyers. Therefore the maximum possible penalties are rarely given for the charge of possession of illegal wildlife products. More often, and as occurred in this case, an agreement is reached between the lawyers. Here, the culprits were released on their own recognizance after they promised to donate US$ 200 to a non- governmental organization dedicated to conservation. However, recent changes in Chile laws are being implemented, which will likely address some of the loopholes in current regulations (i.e. new CITES Law N° 20962).

Molecular genetic approaches are also improving rapidly. Next Generation Sequencing approaches are being designed and implemented that will more accurately identify and distinguish species, populations, and individuals of wildlife species [39] and will become powerful tools to authenticate the origin of products in markets [40–42], using specific markers throughout the genome which includes the functional genetic variation [43]. These approaches promise to provide practical tools to combat wildlife crime more rapidly and lower costs compared to traditional analyses [3,5,44]. In the future, the development and implementation of SNP panels will facilitate more-refined differentiation among wild, domestic and hybrid forms. For now, coat color gene analyses provide useful, cost-effective and accessible approaches for resolving these types of forensic/legal cases.

CRediT authorship contribution statement

Benito A. González: Conceptualization, Investigation, Supervision, Validation, Writing - original draft, Writing - review & editing. **Ana María Agapito:** Data curation, Formal analysis, Software, Writing - original draft. **Francisco Novoa-Muñoz:** Formal analysis, Software. **Juliana Vianna:** Investigation, Resources, Supervision, Visualization, Writing - review & editing. **Warren E. Johnson:** Investigation, Validation, Writing - review & editing. **Juan Carlos Marín:** Conceptualization, Funding acquisition, Investigation, Resources, Supervision, Visualization, Writing - original draft, Writing - review & editing.

Acknowledgements

The authors gratefully acknowledge the assistance of Ximena Contreras from the Servicio Agrícola y Ganadero (Agricultural and Livestock Service) and Loreto González from the Cruz de Piedra Ranch, GASCO S.A. This work was funded entirely by Laboratorio de Genómica y Biodiversidad, Universidad del Bío-Bío. BAG finished this manuscript during a stay at the University of Durham, UK, under the project REDI-170208. WEJ prepared portions of this manuscript while holding a National Research Council Research Associateship Award at the Walter Reed Army Institute of Research and the published material reflects the views of the authors and should not be construed to represent those of the USA Department of the Army or the Department of Defense.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.fsigen.2019.102226.

References

[1] S. Pascoe, T.A. Okey, S. Griffiths, Economic and ecosystem impacts of illegal, unregulated and unreported (IUU) fishing in Northern Australia, Aust. J. Agric. Resour. Econ. 52 (2008) 433–452.

[2] Y. Moodley, I.-R.M. Russo, D.L. Dalton, A. Kotzé, S. Muya, P. Haubensak, B. Bálint, G.K. Munimanda, C. Deimel, A. Setzer, K. Dicks, B. Herzig-Straschil, D.C. Kalthoff, H.R. Siegismund, J. Robovský, P. O'Donoghue, M.W. Bruford, Extinctions, genetic erosion and conservation options for the black rhinoceros (*Diceros bicornis*), Sci. Rep. 7 (2017) 41417.

[3] R. Ogden, N. Dawnay, R. McEwing, Wildlife DNA forensics—bridging the gap between conservation genetics and law enforcement, Endanger. Species Res. 9 (2009)

179–195.
[4] A. Iyengar, Forensic DNA analysis for animal protection and biodiversity conservation: a review, J. Nat. Conserv. 22 (2014) 195–205.
[5] R. Ogden, A. Linacre, Wildlife forensic science: a review of genetic geographic origin assignment, Forensic Sci. Int. Genet. 18 (2015) 152–159.
[6] J.C. Marín, C.E. Saucedo, P. Corti, B.A. González, Application of DNA forensic techniques for identifying poached guanacos (*Lama guanicoe*) in Chilean Patagonia, J. Forensic Sci. 54 (2009) 1073–1076.
[7] D.E. Kane, R.S. Hellberg, Identification of species in ground meat products sold on the U.S. commercial market using DNA-based methods, Food Control 59 (2016) 158–163.
[8] M.C. Ball, L.A. Finnegan, T. Nette, H.G. Broders, P.J. Wilson, Wildlife forensics: “Supervised” assignment testing can complicate the association of suspect cases to source populations, Forensic Sci. Int. Genet. 5 (2011) 50–56.
[9] N. Mucci, C. Mengoni, E. Randi, Wildlife DNA forensics against crime: resolution of a case of tortoise theft, Forensic Sci. Int. Genet. 8 (2014) 200–202.
[10] A. Gentry, J. Clutton-Brock, C.P. Groves, The naming of wild animal species and their domestic derivatives, J. Archaeol. Sci. 31 (2004) 645–651.
[11] P.J.K. McGowan, L. Mair, A. Symes, J.R.S. Westrip, H. Wheatley, S. Brook, J. Burton, S. King, W.J. McShea, P.D. Moehlman, A.T. Smith, J.C. Wheeler, S.H.M. Butchart, Tracking trends in the extinction risk of wild relatives of domesticated species to assess progress against global biodiversity targets, Conserv. Lett. (2018) e12588.
[12] J.C. Wheeler, Evolution and present situation of the South American Camelidae, Biol. J. Linn. Soc. 54 (1995) 271–295.
[13] J.C. Marín, B. Zapata, B.A. González, C. Bonacic, J.C. Wheeler, C. Casey, Sistemática, taxonomía y domesticación de alpacas y llamas: nueva evidencia cromosmica y molecular, Rev. Chil. Hist. Nat. 80 (2007) 121–240.
[14] R.B. Baldi, P. Acebes, E. Cuéllar, M. Funes, D. Hoces, S. Puig, W.L. Franklin, *Lama guanicoe*. The IUCN Red List of Threatened Species, (2016) e.T11186A18540211.
[15] N. Soto, O. Skewes, B.A. González, Conservación y manejo del guanaco en Magallanes, Chile: desde la recuperación poblacional a la revalorización mediante cosecha, GECS News 8 (2018) 35–47.
[16] A. Rosser, M. Haywood, Guidance for CITES Scientific Authorities: Checklist to Assist in Making Non-Detriment Findings for Appendix II Exports, IUCN, Gland, Switzerland and Cambridge, UK, 2002 N° 27. xi + 146pp.
[17] J.C. Marín, R. Rivera, V. Varas, J. Cortés, A. Agapito, A. Chero, A. Chávez, W.E. Johnson, P. Orozco-terWengel, Genetic variation in coat colour genes MC1R and ASIP provides insights into domestication and management of South American camelids, Front. Genet. 9 (2018) 487.
[18] J.C. Marin, B.A. González, E. Poulin, C.S. Casey, W.E. Johnson, The influence of the arid Andean high plateau on the phylogeography and population genetics of guanaco (*Lama guanicoe*) in South America, Mol. Ecol. 22 (2013) 463–482.
[19] S.F. Altschul, W. Gish, W. Miller, E.W. Myers, D.J. Lipman, Basic local alignment search tool, J. Mol. Biol. 215 (1990) 403–410.
[20] N.L. Feeley, K.A. Munyard, Characterisation of the melanocortin-1 receptor gene in alpaca and identification of possible markers associated with phenotypic variations in colour, Anim. Prod. Sci. 49 (2009) 675–681.
[21] N.L. Feeley, S. Bottomley, K.A. Munyard, Three novel mutations in ASIP associated with black fibre in alpacas (*Vicugna pacos*), J. Agric. Sci. 149 (2011) 529–538.
[22] P. Librado, J. Rozas, DnaSP v5: a software for comprehensive analysis of DNA polymorphism data, Bioinformatics 25 (2009) 1451–1452.
[23] S.R. Browning, B.L. Browning, Rapid and accurate haplotype phasing and missing-data inference for whole-genome association studies by use of localized haplotype clustering, Am. J. Hum. Genet. 81 (2007) 1084–1097.
[24] K.D.M. Lang, Y. Wang, Y. Plante, Fifteen polymorphic dinucleotide microsatellites in llamas and alpacas, Anim. Genet. 27 (1996) 293.
[25] M.C.T. Penedo, A.R. Caetano, K.I. Cordova, Microsatellite markers for South American camelids, Anim. Genet. 29 (1998) 411–412.
[26] M.C.T. Penedo, A.R. Caetano, K. Cordova, Eight microsatellite markers for South American camelids, Anim. Genet. 30 (1999) 166–167.
[27] R.J. Sarno, V.A. David, W.L. Franklin, S.J. O'Brien, W.E. Johnson, Development of microsatellite markers in the guanaco, Lama guanicoe: utility for South American Camelids, Mol. Ecol. 9 (2000) 1922–1924.
[28] S.D.E. Park, Trypanotolerance in West African Cattle and the Population Genetic Effects of Selection, University of Dublin, 2002.
[29] G. James, D. Witten, T. Hastie, R. Tibshirani, An Introduction to Statistical Learning: With Applications in R, Springer, New York, 2013.
[30] R Core Team, R: a Language and Environment for Statistical Computing, R Foundation for Statistical Computing, Vienna, Austria, 2013.
[31] E.C. Anderson, Assessing the power of informative subsets of loci for population assignment: standard methods are upwardly biased, Mol. Ecol. Resour. 10 (2010) 701–710.
[32] I.W. Evett, B.S. Weir, Interpreting, D.N.A. Evidence: Statistical Genetics for Forensic Scientists, Sinauer, MA (1998), pp. 142–146.
[33] B.A. González, ¿El guanaco está de vuelta? Dedal de Oro 63 (2013) 34–35.
[34] J. Sambrook, D.W. Russell, Purification of nucleic acids by extraction with phenol: chloroform, Cold Spring Harb. Protoc. (2006) 1 pdb-prot 4455.
[35] J.C. Marín, K. Romero, R. Rivera, W.E. Johnson, B.A. González, Y-chromosome and mtDNA variation confirms independent domestications and directional hybridization in South American Camelids, Anim. Genet. 48 (2017) 591–595.
[36] S.M. Haig, T.D. Mullins, E.D. Forsman, P.W. Trail, L. Wennerberg, Genetic identification of spotted owls, barred owls, and their hybrids: legal implications of hybrid identity, Conserv. Biol. 18 (2004) 1347–1357.
[37] R. Lorenzini, P. Cabras, R. Fanelli, G.L. Carboni, Wildlife molecular forensics: identification of the Sardinian mouflon using STR profiling and the Bayesian assignment test, Forensic Sci. Int. Genet. 5 (2011) 345–349.
[38] S. Vargas, P. Castro-Carrasco, N. Rust, J.L. Riveros, Climate Change Contributing to Perceived Livestock-Guanaco Conflict in Central Chile, (2019) Oryx (in press).
[39] R. Ogden, SNP Discovery: next generation sequencing unlocking the potential of genomic technologies for wildlife forensics, Mol. Ecol. Resour. 11 (Suppl. 1) (2011) 109–116.
[40] E.E. Nielsen, A. Cariani, E. Mac Aoidh, G.E. Maes, I. Milano, R. Ogden, M. Taylor, J. Hemmer-Hansen, M. Babbucci, L. Bargelloni, D. Bekkevold, E. Diopere, L. Grenfell, S. Helyar, M.T. Limborg, J.T. Martinsohn, R. McEwing, F. Panitz, T. Patarnello, F. Tinti, J.K.J. Van Houdt, F.A.M. Volckaert, R.S. Waples, FishPopTrace Consortium, G.R. Carvalho, Gene-associated markers provide tools for tackling illegal fishing and false eco-certification, Nat. Commun. 3 (2012) 851.
[41] S. Wilkinson, A.L. Archibald, C.S. Haley, H.-J. Megens, R.P.M.A. Crooijmans, M.A.M. Groenen, P. Wiener, R. Ogden, Development of a genetic tool for product regulation in the diverse British pig breed market, BMC Genomics 13 (2012) 580.
[42] D. Scarano, R. Rao, DNA markers for food products authentication, Diversity 6 (2014) 579–596.
[43] N.J. Ouborg, C. Pertoldi, V. Loeschcke, R.K. Bijlsma, P.W. Hedrick, Conservation genetics in transition to conservation genomics, Trends Genet. 26 (2010) 177–187.
[44] P. de Knijff, From next generation sequencing to now generation sequencing in forensics, Forensic Sci. Int. Genet. 38 (2019) 175–180.