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'ForCyt' DNA database of wildlife species

N. Ahlers[a], J. Creecy[b], G. Frankham[c], R.N. Johnson[c], A. Kotze[d], A. Linacre[e], R. McEwing[f,*], M. Mwale[d], J.J. Rovie-Ryan[g], F. Sitam[g], L.M.I. Webster[h]

[a] TRAFFIC, The Wildlife Trade Monitoring Network, David Attenborough Building, Cambridge, United Kingdom
[b] University of Oklahoma, USA
[c] Australian Centre for Wildlife Genomics, Australian Museum, Sydney, Australia
[d] National Zoological Gardens of South Africa, South Africa
[e] College of Science & Engineering, Flinders University, Australia
[f] TRACE Wildlife Forensics Network, Edinburgh, United Kingdom
[g] National Wildlife Forensic Laboratory, Department of Wildlife and National Parks, Malaysia
[h] Wildlife DNA Forensics Unit, Science and Advice for Scottish Agriculture, Edinburgh, United Kingdom

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ABSTRACT

Wildlife crime continues unabated contributing to the extinction or near extinction of many plant and animal species. Species identification is a key tool in the enforcement of national legislation. If no morphology exists, comparison of DNA sequences generated from a mitochondrial gene are compared to those on a reference database, commonly GenBank. Sequences up-loaded to GenBank are unregulated and can lead to uncertainty with the adequacy of this DNA sequence repository for identification in a forensic context. We propose the establishment of ForCyt as a fully-regulated database of species that are commonly encountered in forensic investigations. The establishment of ForCyt will allow confidence in future species identification; something that is an absolute requirement to ensure high quality forensic science.

1. Introduction

Substantive evidence now supports a sixth period of wildlife extinction [1] driven by anthropogenic activities and primarily as a result of habitat loss and unsustainable wildlife harvesting for either consumption, perceived medicinal properties, the pet trade or ornamental uses. The Illegal Wildlife Trade (IWT) is a broad term used to identify crimes associated with the illicit exploitation, possession, processing, transportation, and sale of protected flora and fauna, as well as concealment or laundering of the financial benefits from these activities [2]. However, internationally, the laws to tackle IWT are often outdated or inadequate to address the seriousness of some offenses. IWT was until recently considered an emerging threat to ecosystems and the biodiversity they support, but today is now recognised as one of the largest transnational organised criminal activities alongside drug trafficking, arms, and human trafficking [3] with an estimated annual value of between 7 billion and 23 billion US dollars [4].

To aid with enforcement action against wildlife criminals, the discipline of wildlife forensics (scientific analysis of wildlife, their products and derivatives for enforcement purposes), has been developing. This field aims to provide evidence to help prosecute offenders and therefore enforce legislation as well as produce intelligence information with an aim to highlight illegal trade dynamics and disrupt this trade [5]. Similar to its use in human identification, DNA analysis offers a powerful evidential and intelligence gathering tool, however rather than a focus on identifying and linking individuals through nuclear STR profiling, wildlife DNA forensics is predominately utilised to definitively identify a legally protected species, particularly when morphological characters are absent or uninformative. Often the results of species identification are critical in informing the prosecution of the legislation and/or applicable charges. Therefore, the generation of mitochondrial DNA (mtDNA) gene sequences which are appropriate for species identifications from various taxonomic groups, and reference DNA sequences with which to compare case samples is imperative.

Human DNA forensics also utilise information from some mtDNA genes, employing one universal reference sequence to which all variants are compared, although almost exclusively the control region gene when DNA quality is compromised. The Cambridge Reference Sequence (CRS) [6], later the revised rCRS, fills this necessary requirement and ensures harmonisation across the world for human DNA forensic analysis. Regulation and quality checks of mtDNA sequences are essential in forensic science and establishment of EMPOP, a quality controlled

* Corresponding author.
E-mail address: ross.mcewing@tracenetwork.org (R. McEwing).

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database of human mtDNA, is a recognition of this [7].

Despite the reliance on mtDNA for wildlife forensic applications, and broader usage of different mtDNA genes, e.g. cytochrome b [8], cytochrome oxidase I [9] and D-loop/control region [10] among others, there is a paucity of publically available reference data appropriate for forensic applications. This results in heavy reliance on un-validated, non-quality controlled, research-focused public access DNA databases, such as GenBank, which lack any forensic appropriate quality controls and often contain mistakes [9].

2. Project

To address this gap in forensically appropriate resources, the ForCyt project seeks to identify voucher samples of species targeted by wildlife crime not only from a single gene, but from the entire mtDNA genome to be sequenced. These reliable sequence data may act as reference data for that species to enhance the robustness of wildlife forensic applications. By generating the whole mtDNA genome, the reference sequences can be used across all laboratories, even where different mtDNA genes may be used for species identification. The levels of variation among different mtDNA regions could also provide a rich resource for subspecies or population identification if sufficient numbers of vouchers are processed. However, DNA sequence data alone is insufficient as reference standards need additional metadata to ensure species identification is correct, and best practice and quality control reviewing of the data has been followed through the data generation process prior to public release. The use of voucher specimens is in line with the ISFG recommendations [8].

3. Method

As an initial pilot project we have identified some high profile species currently targeted in international illegal wildlife trade (Table 1). Reference samples of each species are currently being collected either across their natural range in Africa or South East Asia or from museums and/or zoological collections with appropriate ancillary information on their provenance. Accurate identification of taxa is key to the success of ForCyt and acceptance of any sample first requires taxonomic identification by a person known as an expert in this field, and further documented evidence to support further identification in the event of a subsequent query. Similarly, identification alone is irrelevant if a chain of custody of sampling process is not also documented, so in addition to a robust documentation pertaining to the identification of the species, an additional affidavit relating to the control of sample processing will also make up the supporting metadata accompanying the DNA sequence files. DNA sequence review, editing and nomenclature will be validated by an expert technical working group and modifications documented as an additional metadata file.

Mitochondrial genome sequence data will be produced following a modified protocol [11], where DNA is recovered avoiding centrifugation stages to prevent nicking the circular mitochondrial DNA genomes, and where linear (nuclear) DNA is digested using an Exonuclease V enzyme and removed by magnetic bead capture approach. The removal of nuclear DNA to ensure sufficient mtDNA coverage during sequencing has the additional benefit of removing any possible nuclear pseudogenes which can complicate species DNA identification issues. Library preparations will utilise the Nextera® XT Library Prep and Index kit (Illumina®) and samples pooled for subsequent sequencing on a MiSeq (Illumina®) platform targeting approximate 100 fold coverage. Assembly and annotation of the genomes will utilise the ROWIN pipeline which was developed for wildlife forensics and allows for the rapid and accurate assembly and annotation of whole mitochondrial genomes.

Once reviewed, assembled sequences and metadata files will be uploaded to the Open Science Framework as public accessible files, but a ‘front page’ url www.ForCyt.org which will provide direct links and supplemental information for the forensic community utilising the data.

Table 1
Listing the species to be included in ForCyt.

Class	Common name	Scientific name	Red List status	CITES Appendix
Mammalia	Giant ground pangolin	*Smutsia gigantea*	Vulnerable	I
	Ground pangolin	*Smutsia temminckii*	Vulnerable	I
	White bellied pangolin	*Phataginus tricuspis*	Vulnerable	I
	Black bellied pangolin	*Phataginus tetradactyla*	Vulnerable	I
	Sunda pangolin	*Manis javanica*	Critically endangered	I
	Chinese pangolin	*Manis pentadactyla*	Critically endangered	I
	Philippine pangolin	*Manis culionensis*	Endangered	I
	Porcupine	*Genus* spp.	Least concern	N/A
	African lion	*Panthera leo*	Vulnerable	I/II
	Leopard (Africa)	*Panthera pardus*	Vulnerable	I
	Leopard (Asia)	*Panthera pardus*	Vulnerable	I
	Serval	*Leptailurus serval*	Least concern	II
	Cheetah	*Acinonyx jubatus*	Vulnerable	I
	Black footed cat	*Felis nigripes*	Vulnerable	I
	Caracal	*Caracal caracal*	Least concern	I/II
	Tiger	*Panther tigris*	Endangered	I
	Clouded leopard	*Neofelis nebulosa*	Vulnerable	I
	Asiatic black bear	*Ursus thibetanus*	Vulnerable	I
	Sun bear	*Helarctos malayanus*	Vulnerable	I
	Hippopotamus	*Hippopotamus amphibius*	Vulnerable	II
	Warthog	*Phacochoerus africanus*	Least concern	N/A
	Mountain Zebra	*Equus zebra*	Vulnerable	I
	Plains Zebra	*Equus quagga*	Near Threatened	N/A
	Grévy's zebra	*Equus grevyi*	Endangered	II
	Sumatran rhino	*Dicerorhinus sumatrensis*	Critically endangered	I
	Javan rhino	*Rhinoceros sondaicus*	Critically endangered	I
	Black rhinoceros	*Diceros bicornis*	Critically endangered	I
	White rhinoceros	*Ceratotherium simum*	Near threatened	I/II
	Indian rhinoceros	*Rhinoceros unicornis*	Vulnerable	I
	African elephant	*Loxodonta africana*	Vulnerable	I/II
	African forest elephant	*Loxodonta africana (cyclotis)*	Vulnerable	I/II
	Asian elephant	*Elephas maximus*	Endangered	I
Aves	Helmeted hornbill	*Rhinoplax vigil*	Critically endangered	I

Similar to other academic fields, research on wildlife genetics is highly competitive and, as a consequence, access to samples from some species is often difficult with a reluctance to share resources. Also the restrictions on the movements of samples from protected species under the CITES convention [12], which although flouted by those involved in illegal wildlife acts, does cause logistical difficulties for researchers and forensic practitioners. The growing collaborative approach being developed by the ForCyt project aims to facilitate and streamline the wildlife crime enforcement community, particularly from developing countries, e.g. Malaysia and South Africa, to releasing samples and data and working within sample movement constraints to support the global community in tackling wildlife crime.

4. Conclusion

The loss of liberty or character defamation associated with prosecution are extremely serious consequences following criminal prosecution. Therefore, in line with its recognition as one of the largest transnational organised criminal activities globally, the forensic science applied to wildlife crime should be at the highest level of professionalism, quality control and quality assurance. Thus, laboratories undertaking such non-human DNA analysis for evidential requirements in IWT investigations should adhere to appropriate standards and guidelines [8], including the use of appropriate reference standards. For many laboratories, these reference standards are impossible to access, and the ForCyt project aims to advance the field by building the foundations for robust wildlife forensic applications.

Conflicts of interest statements

No authors declared any conflicts of interest.

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References

[1] G. Ceballos, P.R. Ehrlich, R. Dirzo, Biological annihilation via the ongoing sixth mass extinction signalled by vertebrate population losses and declines, PNAS 114 (30) (2017) E6089–E6096.
[2] CITES, https://cites.org/prog/iccwc.php/Wildlife-Crime.
[3] UNODC, World Wildlife Crime Report: Trafficking in Protected Species, (2016).
[4] C. Nellemann, R. Henriksen, P. Raxter, N. Ash, E. Mrema, The Environmental Crime: Crisis? Threats to Sustainable Development from Illegal Exploitation and Trade in Wildlife and Forest Resources, (2014).
[5] R. Ogden, N. Dawnay, R. McEwing, Wildlife DNA forensics – bridging the gap between conservation genetics and law enforcement, Endangered Species Res. 9 (2009) 179–195.
[6] R.M. Andrews, I. Kubacka, P.F. Chinnery, R.N. Lightowlers, D.M. Turnbull, N. Howell, Reanalysis and revision of the Cambridge reference sequence for human mitochondrial DNA, Nat. Genet. 23 (2) (1999).
[7] W. Parson, A. Dür, EMPOP – a forensic mtDNA database, FSI: Genet. 1 (2007) 88–92.
[8] A. Linacre, L. Gusmão, W. Hecht, A. Hellmann, W. Mayr, W. Parson, M. Prinz, P. Schneider, N. Morling, ISFG: Recommendations regarding the use of non-human (animal) DNA in forensic genetic investigations, Forensic Sci. Int. Genet. 5 (5) (2011) 501–505.
[9] N. Dawnay, R. Ogden, R. McEwing, G.R. Carvalho, R.S. Thorpe, Validation of the barcoding gene COI for use in forensic genetic species identification, Forensic Sci. Int. 15 (1) (2007) 1–6 (173).
[10] Y. Ishida, N.J. Georgiadis, T. Hondo, A.L. Roca, Triangulating the provenance of African elephants using mitochondrial DNA, Evol. Appl. 6 (2013) 253–265.
[11] A.D. Jayaprakash, E.K. Benson, S. Gone, R. Liang, J. Shim, L. Lambertini, M.M. Toloue, M. Wigler, S.A. Aaronson, R. Sachidanandam, Stable heteroplasmy at the single-cell level is facilitated by intercellular exchange of mtDNA, Nucleic Acids Res. 43 (4) (2015) 2177–2187.
[12] www.cites.org.