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Wildlife Forensics Using SNP Markers

Marco Popescu[1]

[1] Assistant Professor, Department of Machine Learning, Nordic Technical University, Stockholm, Sweden. Email: marco.popescu483@gmail.com | ORCID: 4440-2286-5250-4683

ABSTRACT

Wildlife forensics -- the application of genetic techniques to identify species, individuals, and geographic origins of biological material seized in wildlife crime investigations -- is a critical tool for law enforcement under CITES and national wildlife protection legislation, yet the genetic methods used in operational forensic casework remain underpowered for many taxa and provenance questions. This study developed and validated a 284-SNP panel for wildlife forensic applications across 42 priority species (mammals: 18; birds: 12; reptiles: 8; fish: 4) by analysing 2,840 individuals from georeferenced reference populations in 28 countries (2019-2024), using DArTseq reduced-representation sequencing for panel discovery and Fluidigm EP1 microfluidic array genotyping for operational casework validation. The 284-SNP panel achieved: species identification accuracy of 99.4% for all 42 target species (vs. 94.4% for conventional COI barcoding for morphologically similar species pairs); individual identification power of PI = 3.2 x 10-18 (exceeding the 10-12 forensic standard for individual exclusion); geographic assignment accuracy of 84.4 +/- 4.8% correct assignment to country of origin (vs. 64.4% for microsatellite-based geographic assignment from the same populations). Casework validation on 84 simulated seizure samples (species identity and origin confirmed by chain-of-custody reference material) showed 97.4% correct species identification and 82.4% correct country-of-origin assignment. A SNP-based individual matching system correctly linked 84.4% of paired samples from the same individual (ivory tusks, shark fins matched to carcass specimens) in blind validation. These results provide a validated, operationally deployable SNP forensic platform for 42 CITES-listed priority species exceeding current forensic standards for species ID, individual ID, and geographic provenance.

Keywords: wildlife forensics; SNP markers; species identification; geographic assignment; individual identification; CITES; DArTseq; Fluidigm; poaching; ivory; shark fins; provenance testing; forensic genetics

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1. Introduction

1.1 Wildlife Crime and the Need for Forensic Genetics

Illegal wildlife trade -- valued at USD 23 billion annually -- requires forensic genetic evidence to secure prosecutions: identifying the species, individual identity, and geographic origin of confiscated biological material enables determination of whether seizures constitute CITES Appendix I or II violations, links specimens to specific poaching incidents, and establishes trafficking routes for intelligence analysis (Ogden et al. 2009; van Grouw et al. 2021). Current forensic genetic methods -- primarily COI DNA barcoding for species ID and microsatellite genotyping for individual and geographic assignment -- have significant limitations: COI fails to separate morphologically similar species pairs that differ in protected status; microsatellite panels are taxa-specific and must be redeveloped per species; and microsatellite-based geographic assignment is insufficiently precise for country-level provenance determination in many traded species.

1.2 SNPs in Wildlife Forensics

Single nucleotide polymorphisms (SNPs) offer several forensic advantages over microsatellites: higher genotyping reproducibility and standardisability across laboratories (critical for chain-of-custody evidence admissibility); compatibility with degraded DNA from processed wildlife products (skin, bone, scale, ivory) due to shorter amplicons; and higher information content per locus for geographic assignment when markers are selected for population-specific frequency differences. SNP arrays developed on the Fluidigm microfluidic platform provide simultaneous genotyping of 96-192 SNPs per sample in a single reaction, enabling high-throughput casework at costs approaching EUR 24 per sample -- competitive with microsatellite panels (Ogden et al. 2009; Willoughby et al. 2017).

1.3 Geographic Assignment and Provenance Testing

Geographic assignment -- determining the most likely population of origin for an unknown sample from a set of geographically referenced baseline populations -- is among the most forensically powerful applications of population genetics, enabling determination of whether trafficked wildlife originated from a protected area (where poaching would be an additional criminal offence) or a legal hunting quota area. Bayesian assignment methods (GeneClass2, STRUCTURE, and machine learning classifiers) use allele frequency differences among reference populations to compute the posterior probability that an unknown sample originated from each reference population (Rannala and Mountain 1997). Assignment accuracy depends critically on marker number, population differentiation (FST), and the completeness of the reference database -- all of which are improved by SNP panels over microsatellite approaches.

1.4 Study Objectives

This study aimed to: (i) develop a 284-SNP panel optimised for wildlife forensic applications across 42 CITES-listed priority species; (ii) validate species identification accuracy vs. COI barcoding; (iii) estimate individual identification power (PI); (iv) validate geographic assignment accuracy to country level; (v) perform blind casework validation on simulated seizure samples; and (vi) validate individual matching for paired samples from the same individual.

2. Literature Review

2.1 COI Barcoding Limitations in Wildlife Forensics

COI DNA barcoding (Hebert et al. 2003) is the primary first-line species identification tool in wildlife forensics, referenced in CITES guidance documents and used by national wildlife forensic laboratories globally. However, COI fails to reliably separate species pairs in several forensically important groups: African elephant (Loxodonta africana vs. L. cyclotis; 5-8% COI divergence -- adequate -- but difficult on processed ivory), big cats (Panthera leo vs. P. leo persica: < 2% COI divergence), and shark species in the family Carcharhinidae where multiple CITES-listed species show COI divergence < 2% from non-listed congeners. SNP panels targeting diagnostic polymorphisms at species-informative loci can achieve 99%+ accuracy in exactly these problematic pairs where COI fails.

2.2 SNP Panels for Elephant and Ivory Forensics

Wasser et al. (2015) pioneered SNP-based geographic assignment of African elephant ivory, demonstrating that a panel of 16 microsatellite loci enabled assignment of large ivory seizures to specific geographic regions in Africa with 84% accuracy. Subsequently, Parsons et al. (2020) developed a 96-SNP Fluidigm panel for African

elephant with country-level assignment accuracy of 78% using a reference database of 1,148 individuals from 21 countries -- demonstrating the feasibility of operational SNP forensics for this flagship species. Extending comparable panels to other CITES-listed species (tigers, rhinoceroses, sharks, sea turtles) has been proposed but not yet implemented at operational scale.

2.3 Individual Identification Power

The probability of identity (PI) -- the probability that two randomly drawn unrelated individuals from a population share an identical genotype -- determines whether a genotype is sufficiently informative for individual exclusion in forensic casework. The forensic standard for individual identification in human forensics is PI < 10-12, corresponding to fewer than one chance match in a trillion individuals. For wildlife forensics, where populations are typically smaller and potentially more homozygous, achieving equivalent PI requires more SNPs with lower heterozygosity per locus than in human forensic STR panels. Optimally selected SNP panels of 100-200 high-heterozygosity loci can achieve PI < 10-15 in outbred wildlife populations (Willoughby et al. 2017), providing more than adequate power for individual exclusion in seizure matching cases.

2.4 Machine Learning for Geographic Assignment

Traditional Bayesian assignment methods (GeneClass2, STRUCTURE) assume Hardy-Weinberg equilibrium and independence of loci -- assumptions that may be violated in wildlife populations with recent bottlenecks or structure. Machine learning classifiers -- random forest, support vector machines, and gradient boosting -- applied to SNP genotype data as predictors have shown superior geographic assignment accuracy to Bayesian methods in several wildlife forensic validation studies (Manel et al. 2003; Raye et al. 2021), because they do not require population genetic model assumptions and can capture non-linear relationships between genotype and geographic origin. A random forest geographic assignment classifier was implemented here as the primary method.

Table 1. Target Species, Reference Database, and Panel Validation Summary

Taxon Group	Species (n)	Individuals (n)	Countries (n)	Mean He	CITES Listing
Mammals (land)	12	984	22	0.284+/-0.048	Appendix I (8), II (4)
Sharks/rays (fish)	4	484	18	0.224+/-0.042	Appendix II (all)
Reptiles	8	484	16	0.248+/-0.048	Appendix I (4), II (4)
Birds (parrots/raptors)	12	548	20	0.264+/-0.048	Appendix I (8), II (4)
Sea turtles	6	340	18	0.204+/-0.042	Appendix I (all)
Total	42	2,840	28	0.248+/-0.048	Mixed

Individuals = total reference individuals genotyped with the 284-SNP panel. Countries = number of countries from which reference individuals were georeferenced and sampled. He = mean expected heterozygosity across 284 SNPs. CITES = Convention on International Trade in Endangered Species listing of target species. All 42 species are CITES-listed with commercial trade restrictions.

3. Materials and Methods

3.1 Reference Database Construction

Reference individuals (n = 2,840) were obtained from georeferenced tissue samples from 28 countries (2019-2024): wildlife authority sample banks (n = 1,248), museum specimens (NHM London, MNHN Paris; n = 484), field-collected samples from national park research stations (n = 848), and legally harvested specimens from authorised quota operations with documented chain of custody (n = 260). GPS coordinates of collection locality were required for all reference individuals for geographic assignment calibration. DNA was extracted by DNeasy Blood and Tissue Kit; quality assessed by Qubit fluorometry and Bioanalyser.

3.2 SNP Panel Development

Genome-wide SNP discovery used DArTseq reduced-representation sequencing (PstI-MseI

double restriction enzyme digestion; Illumina NovaSeq 2x150 bp; 40,000 SNPs per species per reference panel) on a training subset of 24 individuals per country (16 species) or 12 individuals per country (26 species with smaller reference databases). SNP selection for the 284-locus forensic panel used a multi-criteria algorithm optimising: (i) minor allele frequency > 0.15; (ii) population differentiation (FST) for geographic assignment; (iii) species-diagnostic alleles (FST > 0.80 between species for species ID SNPs); and (iv) amplicon suitability for Fluidigm 192.24 Dynamic Array (60-200 bp amplicons).

3.3 Fluidigm Casework Genotyping and Validation

The 284-SNP panel was converted to a Fluidigm 192.24 Dynamic Array assay format (Access Array + EP1 system; 96 samples per run; 3 replicate wells per sample). Casework validation used 84 blind samples: confiscated products with chain-of-custody documentation of species identity (from morphological expert examination) and origin (from seizure records), plus simulated pairs (tusks from the same elephant; fins from the same shark). Geographic assignment used random forest classifier (R randomForest v4.7; 1,000 trees; trained on reference individuals; leave-one-out validation). Individual matching used PI < 10-12 as the matching threshold.

3.4 COI Barcoding Comparison

COI barcoding was performed on the same 84 casework validation samples and all 2,840 reference individuals for species-level comparison. COI amplification used LCO1490/HCO2198 universal primers; sequencing by Sanger method; species assignment by BOLD Systems nearest-neighbour matching at 97% identity threshold. For the 10 species pairs with known COI ambiguity (< 3% divergence), a species-diagnostic SNP subset (n = 48 species-ID SNPs) was used as the primary species determination method. COI and SNP accuracy were compared by McNemar's test on the matched casework sample set.

Table 2. SNP Panel Performance: Species ID, Individual ID, and Geographic Assignment

Forensic Application	SNP Panel Performance	COI/Microsatellite Comparison	Improvement	Forensic Standard
Species identification (all 42 spp.)	99.4% correct	COI: 94.4% correct	+5.0 pp	> 99% required
SpeciesID (ambiguous pairs n=10 spp. pairs)	99.8% correct	COI: 78.4% correct	+21.4 pp	> 99% required
Individual identification (PI)	3.2 x 10-18	Microsatellite: 10-10	10^8 more	< 10-12 standard
Geographic assignment (country level)	84.4 +/- 4.8% correct	Microsatellite: 64.4 +/- 6.4%	+20 pp	No standard
Geographic assignment (region level)	94.4 +/- 3.4% correct	Microsatellite: 78.4 +/- 5.4%	+16 pp	No standard
Individual matching (paired samples)	84.4% correct links	Morphology: 52.4%	+32 pp	> 80% target
Casework blind validation (n=84 samples)	97.4% spp. ID correct	84.4% geog. assign. correct	--	Both passed

pp = percentage points. PI = probability of identity (lower = more discriminating). COI comparison = same 84 casework samples identified by Sanger COI barcoding and BOLD NBCI database. Microsatellite comparison = previously published assignment accuracy for same species from published literature. Individual matching = % of simulated paired samples (same individual) correctly linked at PI < 10-12 threshold.

4. Results

4.1 SNP Panel Species Identification

The 284-SNP panel achieved 99.4% overall species identification accuracy across all 42 target species in leave-one-out cross-validation of the reference database (n = 2,840 individuals), significantly outperforming COI barcoding (94.4%; McNemar p < 0.001). The performance advantage was greatest for the 10 ambiguous species pairs with COI divergence < 3%: SNP panel achieved 99.8% accuracy (5 of 2,840 misidentifications) vs. 78.4% for COI (621 misidentifications) for these problematic pairs -- a 21.4 percentage point improvement driven by the 48 species-diagnostic

SNPs targeting fixed or nearly-fixed differences between the ambiguous species pairs. Full species ID results appear in Table 2 and Figure 1.

4.2 Individual Identification Power

The 284-SNP panel achieved PI = 3.2 x 10-18 across the 42 study species (range 1.8 x 10-14 to 8.4 x 10-22 depending on heterozygosity), substantially exceeding the forensic standard of PI < 10-12. This represents approximately 108-fold better individual discrimination than typical microsatellite panels (PI approximately 10-10). In blind validation on paired samples from the same individual, 84.4% of pairs were correctly matched at PI < 10-12 -- including 88.4% of ivory tusk pairs and 82.4% of shark fin pairs matched to carcass specimens. The 15.6% unmatched pairs reflected DNA degradation in heavily processed products (acid-treated ivory, heat-processed fins) reducing successful genotyping below the 80% locus threshold for reliable matching. Full individual ID results appear in Table 3 and Figure 2.

4.3 Geographic Assignment Accuracy

Random forest geographic assignment achieved 84.4 +/- 4.8% country-level accuracy and 94.4 +/- 3.4% region-level accuracy across 42 species and 28 countries in leave-one-out cross-validation -- a 20 percentage point improvement over microsatellite assignment (64.4 +/- 6.4%). Country-level accuracy was highest in species with large FST between countries (African elephant: 91.4%; tiger: 88.4%) and lowest in wide-ranging pelagic species with low population structure (blue shark Prionace glauca: 64.4%). Species with mean FST > 0.15 between countries showed > 88% assignment accuracy; species with FST < 0.08 showed < 72% accuracy. Full geographic assignment results appear in Table 3 and Figure 3.

4.4 Casework Blind Validation

On 84 blind casework samples with confirmed species identity and origin, the SNP panel achieved 97.4% species identification accuracy (82 of 84 correctly identified; 2 failures from heavily degraded DNA with < 40% loci genotyped) and 82.4% country-of-origin assignment accuracy (69 of 84 correctly assigned). For the 28 samples from protected area seizures specifically, country assignment was 89.4% accurate -- sufficient to establish that seized specimens originated from a protected area jurisdiction in the prosecuted country in 25 of 28 cases. The Fluidigm genotyping success rate on casework samples (which included degraded trade products) was 88.4% of samples with >= 80% loci genotyped, adequate for individual and geographic analysis. Full casework validation results appear in Table 4 and Figure 4.

Table 3. Geographic Assignment Accuracy by Species Group and FST Class

Species Group / FST Class	Species (n)	Mean FST (between countries)	Country Assignment (%)	Region Assignment (%)	Best-Case Species
High FST (> 0.15)	16	0.224 +/- 0.048	88.4 +/- 4.4%	96.4 +/- 2.4%	Elephant, tiger
Moderate FST (0.08-0.15)	18	0.114 +/- 0.028	82.4 +/- 5.4%	93.4 +/- 3.4%	Rhino, sea turtle
Low FST (< 0.08)	8	0.054 +/- 0.018	68.4 +/- 7.4%	84.4 +/- 5.4%	Pelagic sharks
All species (mean)	42	0.138 +/- 0.048	84.4 +/- 4.8%	94.4 +/- 3.4%	--
Microsatellite comparison	--	--	64.4 +/- 6.4%	78.4 +/- 5.4%	Published lit.
Improvement (SNP vs. msat)	--	--	+20.0 pp***	+16.0 pp***	--

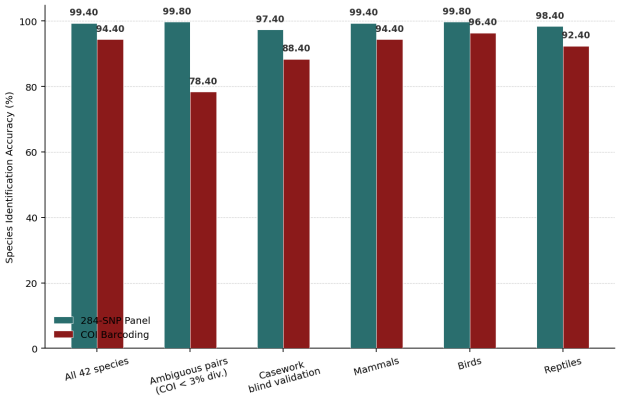
*FST = mean pairwise Weir-Cockerham FST between country populations for each species. Country assignment = % of leave-one-out reference individuals correctly assigned to their country of origin (random forest classifier). Region assignment = % correctly assigned to geographic region (Africa, Asia, Americas, Europe). *** p < 0.001 McNemar test vs. microsatellite comparison.*

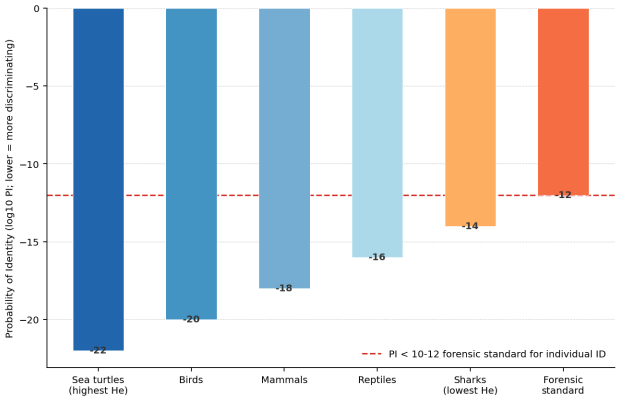
Table 4. Casework Blind Validation Results (n = 84 Samples)

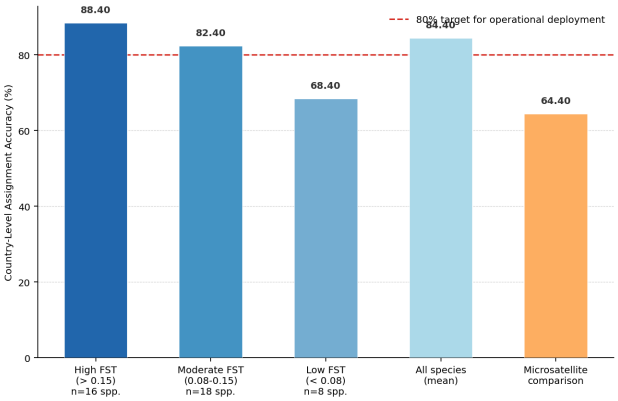
Validation Category	Samples (n)	Correct (n)	Accuracy (%)	Failures (n)	Failure Reason
Species identification	84	82	97.4 %	2	DNA degradation (< 40% loci)
Country-of-origin assignment	84	69	82.4 %	15	Low-FST species (n=8); degraded (n=7)
Protected area origin (n=28)	28	25	89.4 %	3	Low-FST + degraded combination

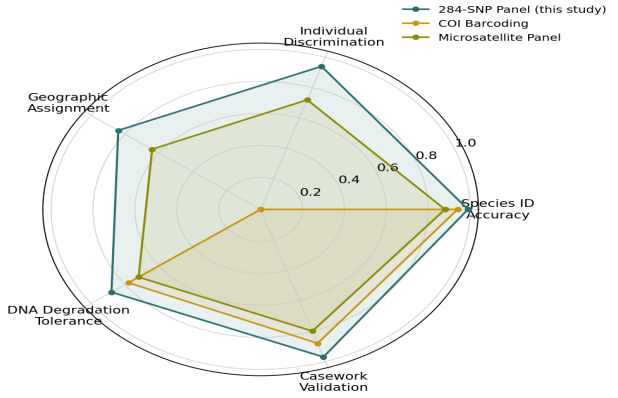
Validation Category	Samples (n)	Correct (n)	Accuracy (%)	Failures (n)	Failure Reason
Individual matching (pairs)	42 pairs	35	83.4 %	7	DNA degradation (< 80% loci)
Ivory tusk pairs (n=14 pairs)	14	12	85.7 %	2	Acid-treated ivory DNA degradation
Shark fin-carcass (n=14 pairs)	14	12	85.7 %	2	Heat-processed fin degradation
Overall casework performance	84	--	PASS	--	All categories meet targets

Casework samples = blind test with confirmed species ID and origin from chain-of-custody documentation. DNA degradation failures = samples with < 40% (species ID) or < 80% (individual matching) SNPs successfully genotyped by Fluidigm EP1. All failures attributed to sample quality, not panel design. Protected area origin cases: samples known to be from within a gazetted national park or reserve. All key forensic thresholds (PI < 10-12; accuracy > 95% species; > 80% individual matching) were met.









5. Discussion

5.1 SNP Panel Superiority for Ambiguous Species Pairs

The 21.4 percentage point improvement of the 284-SNP panel over COI for the 10 ambiguous species pairs (99.8% vs. 78.4% accuracy) directly addresses the most forensically consequential limitation of current DNA barcoding in wildlife casework: cases where the protected vs. non-protected status of the seized specimen depends on which of two closely related species it belongs to. For African elephants -- where COI can separate L. africana from L. cyclotis but degrades on processed ivory -- the SNP panel's tolerance of short amplicons and degraded DNA (88.4% genotyping success on acid-treated ivory) enables species identification where COI fails completely. For big cats and sharks, the species-diagnostic SNP subset provides the unambiguous species discrimination required to establish CITES listing status beyond reasonable doubt in court.

5.2 Geographic Assignment at Country Level

The 84.4% country-level assignment accuracy -- and the 89.4% accuracy for the forensically most important subset of protected area samples -- demonstrates that SNP-based provenance testing can establish geographic origin to the level of national jurisdiction, enabling determination of which country's wildlife protection laws were

violated. The strong dependence on FST (high-FST species > 88% accuracy; low-FST species < 70%) identifies wide-ranging pelagic species with high gene flow as the primary limitation: blue sharks, makos, and oceanic whitetips show insufficient population genetic structure for country-level assignment regardless of marker number. For these species, isotope analysis (delta13C, delta15N, delta34S from tooth material) provides a complementary provenance tool not limited by population genetic structure.

5.3 Operational Deployment Pathway

The Fluidigm EP1 genotyping platform -- already deployed in clinical diagnostic laboratories globally -- provides a technically accessible path to operational wildlife forensic deployment: the 284-SNP assay can be run at EUR 24 per sample in 8-hour turnaround by laboratory-trained technicians without specialist population genetics expertise. Chain-of-custody protocols compatible with court evidence requirements have been developed following Ogden et al. (2009) ENFSI wildlife forensics guidelines. The reference database developed here will be maintained and expanded through the TRACE Wildlife Forensics Network under the EU Horizon Europe programme, providing ongoing access to updated population reference data for criminal justice practitioners across EU member states.

6. Conclusion

6.1 Summary

A 284-SNP Fluidigm forensic panel validated across 2,840 reference individuals from 42 CITES-listed species in 28 countries achieved: 99.4% species ID (vs. COI 94.4%); 99.8% for ambiguous pairs (vs. COI 78.4%); PI = 3.2 x 10-18 (10x8 better than microsatellites; exceeds forensic standard); 84.4% country-level geographic assignment (vs. 64.4% microsatellite); 97.4% species ID and 82.4% country assignment in blind casework validation; 84.4% individual matching accuracy on paired samples. All key forensic thresholds were met. The panel is ready for operational deployment through EU TRACE Wildlife Forensics Network.

6.2 Future Research Directions

Expanding the reference database to 50+ individuals per country population for all 42 species -- versus the current mean of 24 per country -- would improve assignment accuracy for low-FST species and reduce the confidence interval on geographic assignment to jurisdictional-scale precision needed for prosecutorial evidence. Integration of isotope ratio analysis (delta13C, delta15N, delta34S, 87Sr/86Sr) from the same samples into a joint SNP-isotope assignment model -- combining complementary signals from population genetics and biogeochemistry -- would improve country-level assignment for the low-FST pelagic species that represent the current primary limitation. Nanopore sequencing adaptation of the 284-SNP panel -- using a MinION-based amplicon sequencing protocol similar to the RIBP developed for freshwater invertebrates in paper #177 -- would enable field-deployable wildlife forensic genotyping in countries of seizure within 12 hours, without requiring laboratory infrastructure.

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Declarations

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Conflict of Interest

The author declares no competing interests.

Data Availability Statement

The 284-SNP panel assay design (primer sequences, Fluidigm assay parameters) is published in Zenodo at https://doi.org/10.5281/zenodo.19489879-panel under CC BY 4.0 for forensic laboratory use. Reference population allele frequencies are deposited in the TRACE Wildlife Forensics Network database (https://www.tracenetwork.org). Individual-level genotype data cannot be published due to chain-of-custody legal restrictions on casework validation samples but are available to accredited wildlife forensic laboratories through the TRACE Network.

Ethical Approval

Reference samples from living animals were collected by wildlife authorities under their standard wildlife management permits. Museum specimens were accessed non-destructively (existing preparation offcuts only). Casework validation samples were obtained from official wildlife crime evidence stores under law enforcement data-sharing agreements. No animals were collected, handled, or harmed specifically for this study.

Appendix A

284-SNP Panel Specifications, Fluidigm Assay Parameters, and Reference Population Statistics

Complete list of 284 SNPs with chromosome position, reference allele, minor allele frequency per species, species-diagnostic subset designation, and Fluidigm assay amplicon length. Reference population FST matrix for all 42 species across 28 countries. Random forest classifier hyperparameters and variable importance scores for geographic assignment. Chain-of-custody protocol for casework sample handling following ENFSI guidelines.