

SWFS Standards and Guidelines for Wildlife Forensic Analysis - Version 3

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1.0 Scope

This document provides minimum standards and additional guidelines for wildlife forensic analysts in the sub disciplines of DNA (section 4), morphology (section 5), and chemical analysis for timber identification (section 6). This document also covers good laboratory practices, evidence handling, and training, which are central to all forensic laboratories. It also includes critical considerations of phylogeny, taxonomy, and reference collections that are specific to wildlife forensic science.

2.0 Definitions

Note: These definitions apply to all Standards and Guidelines. Specific definitions, where relevant, will be located in those respective sections.

- 2.1 **Accuracy** – The ability to obtain a correct result, e.g., the degree of conformity of a measured quantity to its actual (true) value.
- 2.2 **Administrative Review** – An evaluation of the report and supporting documentation for consistency with laboratory policies and for editorial correctness.
- 2.3 **Analyst** – An individual who conducts and/or directs the analysis of forensic casework samples, interprets data, reaches conclusions, and/or issues reports concerning conclusions.
- 2.4 **Analytical Plan** – A plan for the analytical methods to be applied in a case, dependent on the forensic question, available technologies, preservation of the evidence and the value of the analytical results. Typically documented as laboratory-specific SOPs (see below). All non-standard analytical plans (e.g., for work with novel evidence types) need to be documented in the casefile.
- 2.5 **Chain of Custody** – The chronological documentation or paper trail, showing the seizure, custody, control, transfer, analysis, and disposition of evidence.
- 2.6 **Competency** – The demonstration of technical skills and knowledge necessary to perform certain tasks.
- 2.7 **Curated Collection** – An assemblage of reference materials acquired and maintained with associated data according to explicit quality control standards.
- 2.8 **Guidelines** – these are not mandatory, but represent a “best-case-scenario” for analysts and laboratories with the means to achieve them. Laboratories that encounter forensic casework occasionally may not be able to implement all guidelines. However,

dedicated wildlife forensic laboratories should consider implementation of the guidelines.

- 2.9 **Known** – In the context of evidence, the material for which the character under investigation (e.g., individual identity, geographic source) is unquestioned. This serves as the basis for comparison to questioned material for the purpose of individual matching.
- 2.10 **Identification** – Analyses to establish the taxonomic classification of the sample. These analyses are based on class characters diagnostic for the taxonomic level in question.
- 2.11 **Individualization** – Analyses that attempt to match a questioned to a known sample to the exclusion of all others.
- 2.12 **Laboratory** –The entity providing the analysis, including the staff and the physical facility.
- 2.13 **Precision** – The degree of mutual agreement among a series of individual measurements, values, and/or results.
- 2.14 **Reference Material** – Biological specimens of known identity or data derived from them, or from published sources. Voucher specimens are a subset of reference material which are of known identity, curated with relevant data such as geographical origin, life history stage, and sex.
- 2.15 **Standard Operating Procedure (SOP)** – Written documentation maintained by the laboratory including laboratory policies, technical procedures and protocols or analytical methods for specific forensic procedures. SOPs are controlled documents with mechanisms to assure that content is current and authorized, that previous or outdated versions are archived for reference, and that the SOPs are implemented in the laboratory.
- 2.16 **Standards** – Mandatory minimum practices necessary to ensure that analysts produce accurate, precise analytical findings, and convey these findings in an unbiased, objective manner. Some standards are accompanied by methods for evaluating accuracy and objectivity, e.g., tracking performance of reagents and equipment, or through technical review of analytical products and reports. Standards are non-negotiable, and every analyst shall abide by them whether in a research laboratory or a dedicated forensic facility. Standards and guidelines can be modified in response to new information, innovations, and perspectives.
- 2.17 **Technical Review** – An evaluation of reports, case notes, data, and other documents to ensure there is an appropriate and sufficient basis for the scientific conclusions.
- 2.18 **Validation** – The process of performing a set of experiments that establishes the reliability of a technique or procedure or modification thereof. Method validation demonstrates that an analytical method is acceptable for its intended purpose.

3.0 General Standards and Guidelines

3.1 Training and Personnel

- 3.1.1 *Standard:* Each laboratory shall have a Standard Operating Procedure (SOP) for the training of both experienced and inexperienced workers, incorporating the standards described below.
- 3.1.2 *Standard:* Each laboratory conducting wildlife forensic analyses shall have an ethical code by which all staff must abide. This shall include an explicit statement that all laboratory staff shall conduct their work in a professional, confidential, and unbiased manner.
- 3.1.3 *Guideline:* All analysts and supervisors should have a documented training program.
- 3.1.4 *Standard:* Before assuming independent duties, all members of the laboratory who handle evidence shall have training that includes:
 - 3.1.4.1 health and safety around biological specimens
 - 3.1.4.2 chain of custody
 - 3.1.4.3 secure transfer, storage, and processing of evidence
- 3.1.5 *Standard:* Before undertaking independent casework in a given method, each analyst shall demonstrate competency in that method, verified by blind testing.
- 3.1.6 *Guideline:* Before undertaking independent casework, training of analysts should include:
 - 3.1.6.1 cognitive bias
 - 3.1.6.2 training in relevant laws
 - 3.1.6.3 expert witness testimony

3.2 Evidence Handling

- 3.2.1 *Standard:* Laboratories shall have Standard Operating Procedures (SOPs) in place to assure evidence integrity during storage, processing, examination, and at all times, addressing:
 - 3.2.1.1 evidence receipt
 - 3.2.1.2 acceptance criteria
 - 3.2.1.3 tracking
 - 3.2.1.4 storage
 - 3.2.1.5 transfer
 - 3.2.1.6 post-analysis disposition
 - 3.2.1.7 prevention of evidence loss
 - 3.2.1.8 prevention of contamination
 - 3.2.1.9 prevention of tampering

- 3.2.2 *Standard:* Evidence and derived data shall be stored and analyzed in a controlled and secure manner at all times.
- 3.2.2.1 Physical evidence shall be maintained in locked storage.
- 3.2.2.2 Digital data shall be stored in a secure, restricted location.
- Note: Controlled access includes locked evidence storage, restrictions to forensic analytical spaces, and digital data protection. Access to evidence by non-forensic personnel should be with escort or under supervision at all times.*
- 3.2.3 *Standard:* A chain of custody shall be maintained.
- 3.2.4 *Standard:* All evidence shall be marked with a unique identifier and the signature or initials of all who handle the evidence.
- 3.2.5 *Standard:* A portion of each evidence sample shall be retained, whenever possible, to enable possible future independent analysis.
- 3.2.6 *Standard:* Evidence subject to significant physical alteration in whole or part to assist identification (e.g., parts removed for molecular analyses, skeletonized) shall be photographed prior to alteration.
- 3.2.7 *Standard:* When physically altering evidence for the purpose of analysis, careful consideration shall be given to the effects the alteration(s) may have on possible subsequent analyses.
- 3.2.8 *Guideline:* If alteration that will affect subsequent analysis is necessary, the pertinent party should be consulted.
- 3.2.9 *Standard:* Separate aliquots/batches of reagents shall be used for research and casework.
- 3.2.10 *Standard:* Research and casework samples shall be physically or temporally separated when processed on the same instrument.

3.3 **Equipment and Methods**

- 3.3.1 *Standard:* Instruments shall have their performance checked before use in analyzing casework samples. This can be accomplished by analyzing representative samples (case-type samples, positive controls) and assessing whether the expected results are achieved. Such performance checks shall occur:
- 3.3.1.1 when a new instrument is brought into service
- 3.3.1.2 thereafter on a regular basis (at least as frequently as indicated by the instrument manufacturer)
- 3.3.1.3 after an instrument has been loaned out
- 3.3.2 *Standard:* Laboratories shall have a Standard Operating Procedure (SOP) in place for all analytical methods, including the validation of new laboratory and data analysis methods.
- 3.3.3 *Standard:* Analytical methods used in casework shall be validated prior to use.

- 3.3.4 *Standard:* Use of an analytical method derived from procedures validated at another laboratory or from a method published in the peer-reviewed literature shall undergo an internal validation. The validation shall be of sufficient rigor and detail to confirm that the expected results of the analysis can be achieved at the testing laboratory before the method is used in casework.
- 3.3.5 *Guideline:* The following validation criteria should be addressed if appropriate:
 - 3.3.5.1 Literature review of the relevant issue. A list of relevant references should be available.
 - 3.3.5.2 Accuracy of the analysis. Accuracy can be determined by analyzing a traceable control sample.
 - 3.3.5.3 Precision of the analysis: Precision can be determined by repeated testing of known samples.
 - 3.3.5.4 Specificity of the analysis: Specificity can be evaluated by the analysis of individuals from related but non-target species or populations, likely contaminant species, or substitute species. Alternative sources (tissue types or substrates) can also be tested.
 - 3.3.5.5 Limitations to accurate interpretation (e.g., contaminants in blood mixtures, substrate, fungal or pathogen contamination, etc.) should be identified and evaluated.
- 3.3.6 *Guideline:* It is important that the plan for laboratory analysis is clear, and where this is not documented in SOPs (e.g., for novel sample-types or questions) a separate analytical plan should be formulated for inclusion in case notes, with any deviations from this plan being fully documented.

3.4 Reference Materials and Collections

- 3.4.1 *Standard:* Laboratories conducting wildlife forensic analyses shall maintain or have access to reference materials in curated collections.
- 3.4.2 *Standard:* Laboratories shall have a SOP covering curation and preservation of each type of biological reference material used for taxonomic identification. Topics to be covered include:
 - 3.4.2.1 Documentation and curation procedures
 - 3.4.2.2 Protection of materials from degradation
 - 3.4.2.3 List of currently used taxonomic authorities
- 3.4.3 *Standard:* Specimens and databases used in casework shall be uniquely identified and documented in the case file.
- 3.4.4 *Standard:* The identity of biological reference material must be verified before it is used in casework. Validation of morphological specimens is made with reference to verified specimens at hand, to specimens in a larger natural history collection (e.g., major museums), or to the professional literature (e.g., taxonomic monographs, identification keys, or field guides).

- 3.4.5 *Standard:* The taxonomic identity of reference material or DNA sequences used for comparison to evidence items, as well as associated data on geographic origin and source, shall be documented in a laboratory catalog or database
- 3.4.6 *Standard:* Taxonomic identification reports shall include currently accepted scientific names.
- 3.4.7 *Standard:* Authoritative sources (published literature or databases) shall be used in determining whether a taxonomic classification is scientifically accepted
- 3.4.8 *Guideline:* Laboratory analysts should be prepared to cite the taxonomic authorities used for all classifications in their reports.
- 3.4.9 *Guideline:* Each analyst should be prepared to address synonymies and other potential taxonomic issues.
- 3.4.10 *Guideline:* Subspecies determination of wild taxa should only be attempted with accurate data concerning geographic origin, and with knowledge of currently-accepted subspecies distributions.

3.5 Case Documentation

- 3.5.1 *Standard:* The case file shall include the following:
 - 3.5.1.1 chain of custody
 - 3.5.1.2 submittal request
 - 3.5.1.3 bench notes
 - 3.5.1.4 location of any electronic data
 - 3.5.1.5 documentation of technical reviews
 - 3.5.1.6 final report
- 3.5.2 *Guideline:* The case file should additionally include any other pertinent documents, such as an analytical plan, raw data files, emails, records of other external communications regarding the case, shipping and receiving documentation, and/or photographic documentation of the evidence or packaging.
- 3.5.3 *Standard:* Details in bench notes shall be sufficient to enable another analyst competent in the reporting subject to repeat the analysis conducted under the same methodology and testing conditions.
- 3.5.4 *Standard:* Assumptions of geographical origin used in taxonomic identification shall be documented in the case file.

3.6 Reporting

- 3.6.1 *Standard:* Reports shall include information on general methods, results, and conclusions. The report shall contain sufficient detail for another expert to be able to ascertain how the analyses were accomplished and conclusions drawn.
- 3.6.2 *Standard:* Technical review: all reports shall be reviewed before issue for technical accuracy by another scientist with demonstrable knowledge and expertise in the reporting subject.
- 3.6.3 *Guideline:* Administrative review: all reports should be reviewed by a qualified person to assure correctness of formatting and editorial content
Note: Ideally, the technical and administrative reviews should be done by different people.
- 3.6.4 *Guideline:* Technical reviews shall be documented in the case file, and changes to early report drafts that affect the interpretations should be fully documented.
- 3.6.5 *Standard:* All reports shall identify the analyst(s) involved in generation and interpretation of forensic data.
- 3.6.6 *Standard:* Terms used in the conclusion, such as “match,” “consistent with,” etc., shall be defined by each reporting laboratory.
- 3.6.7 *Standard:* Statistical tests used to indicate confidence in conclusions, such as random match probabilities or likelihood ratios, shall be reported.

4.0 DNA Standards and Guidelines

Wildlife DNA Analysis is the discipline within wildlife forensics using genetic techniques to identify wildlife parts and products to family, genus, species, population, or individual source. Analysis of genetic characters is the method of choice for individualization and classification when morphological characters are absent, particularly with trace evidence (blood, body fluids), partial organisms (gut piles, crafted items, bones, antlers, horns), degraded or processed tissues (cooked meats, fish filets, timber, Traditional Chinese Medicines).

These Standards and Guidelines refer to general considerations in the application of genetic techniques in analyzing wildlife forensic evidence (e.g., restriction fragment length polymorphisms, single nucleotide polymorphisms, or protein analysis). They also cover specific wildlife DNA analyses currently widely employed, such as DNA sequencing for the identification of class characters, and DNA fragment analysis of short tandem repeats (STRs) and Single Nucleotide Polymorphisms (SNPs) for establishing individual identity. It is expected that these standards and guidelines will continue to evolve as the field develops.

4.1 DNA Definitions and Abbreviations

- 4.1.1 **Analytical Thresholds** – In STR analysis, minimum and maximum peak amplitudes acceptable for peaks intended to be assigned allele designations.
- 4.1.2 **Bin** – In STR analysis, a “window” around the size obtained for each allele (determined for each different species with empirical data).
- 4.1.3 **Contamination** – The unintentional introduction of exogenous DNA into a sample or PCR reaction.
- 4.1.4 **Electropherogram** – A plot of results from an electrophoretic analysis generated by a genetic analyzer.
- 4.1.5 **Extraction Negative Control** – (or Reagent Blank) An analytical control sample that contains no template DNA and is used to monitor contamination from extraction to final fragment or sequence analysis. This control is included in the analysis alongside the questioned and/or known samples.
- 4.1.6 **Genotype** – The genetic constitution of an organism or cell; also refers to the specific allele(s) inherited at nuclear or mitochondrial loci.
- 4.1.7 **Heterozygous** – In STR analysis, alleles that appear as a two-peak pattern and, on average, have similar peak heights relative to each other.
- 4.1.8 **Homozygous** – In STR analysis, alleles that appear as single peaks.
- 4.1.9 **Low Copy Number Analysis** – An analysis to obtain a result from very low quality/quantity samples, for example by using additional PCR cycles, differing reagent concentrations, etc.
- 4.1.10 **Mitochondrial Haplotype** – A DNA sequence that has been identified at a specific mitochondrial DNA region.
- 4.1.11 **PCR** – Polymerase Chain Reaction.

- 4.1.12 **PCR Negative Control** – An analytical control used to detect DNA contamination of the amplification reagents. This control consists of only amplification reagents without the addition of template DNA. This control is included in the analysis alongside the questioned and/or known samples.
- 4.1.13 **PCR Positive Control** – An analytical control sample that is used to determine if the PCR performed properly. This control consists of the amplification reagents and a known DNA sample, and is included in the analysis alongside the questioned and/or known samples.
- 4.1.14 **Peak** – A distinct triangular section of an electropherogram that projects above the baseline. In STR analysis, the designation of a peak as an allele is determined primarily by the parameters set in the equipment’s analytical software.
- 4.1.15 **Peak Height** – (or Peak Amplitude) The point at which the signal intensity of the peak is greatest.
- 4.1.16 **Peak Height Ratios** – In STR analysis, the ratio of the height of the lower peak to the height of the higher peak, expressed as a percentage.
- 4.1.17 **Short Tandem Repeats (STRs)** – (or Microsatellites) Polymorphic fragments of DNA containing a repeated sequence of generally 2-5 nucleotides. STRs are commonly used for individualization, as the number of repeats is typically highly variable in a population.
- 4.1.18 **Single Nucleotide Polymorphism (SNP)** - A specific nucleotide position at a target DNA locus that displays (usually bi-allelic) nucleotide variation within a population. SNPs can be used for species identification, population/regional assignment, and individualization.
- 4.1.19 **Theta (Θ)** – An estimator of Wright’s F_{ST} statistic (NRC, 1996) which is used to represent population genetic structure; incorporated as a correction into match probability equations where population reference data contains multiple subpopulations.

4.2 General DNA Standards and Guidelines

4.2.1 Laboratory

- 4.2.1.1 *Standard:* Labs shall have a SOP to cover the process by which facilities and equipment are cleaned and decontaminated.
- 4.2.1.2 *Standard:* Casework and non-casework related research shall be separated spatially or temporally.
- 4.2.1.3 *Standard:* Areas of the laboratory shall be designated post-PCR and pre-PCR.
- 4.2.1.4 *Standard:* Equipment, PCR products, and supplies shall not be transferred from post-PCR to pre-PCR areas unless decontaminated.

4.2.2 DNA Extraction

- 4.2.2.1 *Standard:* Labs shall have SOPs for all extraction methods used in the laboratory.

- 4.2.2.2 *Standard:* Each DNA extraction set shall include at least one extraction negative control.
 - 4.2.2.3 *Standard:* Extraction of DNA from reference material shall be physically or temporally separated from extraction of DNA from evidence.
 - 4.2.2.4 *Standard:* When multiple evidence items are to be compared for individual matching, e.g., questioned vs. known evidence, the items shall be processed at different times or in different places.
 - 4.2.2.5 *Guideline:* Trace samples should be extracted and amplified before samples with high copy number DNA, and questioned samples should be extracted before related reference material and known samples.
 - 4.2.2.6 *Guideline:* In analyses that are sensitive to template concentration, samples should be quantified prior to amplification.
- 4.2.3 Amplification
- 4.2.3.1 *Standard:* Labs shall have SOPs for all PCR methods routinely used in the laboratory.
 - 4.2.3.2 *Standard:* Primers used shall be documented in the case file.
 - 4.2.3.3 *Standard:* Routinely used primers shall have been validated to delimit the range of acceptable PCR conditions and to evaluate the likelihood of encountering false positives and false negatives.
Note: Depending on the analyses to be conducted, examples of testing could include: varying dilutions of template, reagent concentrations, annealing temperatures, cycle numbers, and examination of a variety of likely species to determine specificity.
 - 4.2.3.4 *Standard:* Each PCR shall include an extraction negative control and PCR negative and positive controls.
 - 4.2.3.5 *Guideline:* A positive control should produce a distinctive genotype, to allow one to readily determine that it is not a source of contamination.
 - 4.2.3.6 *Standard:* PCR negative and positive controls and extraction negative controls shall be analyzed with evidence samples through the final step (e.g. sequencing or fragment size determination).
- 4.2.4 Analysis and Interpretation
- 4.2.4.1 *Standard:* The results shall be rejected if a negative control shows amplification and the genotype is identical to an evidence sample.
 - 4.2.4.2 *Standard:* Laboratories shall have SOPs to address the following:
 - 4.2.4.2.1. Contamination detected in positive controls, negative controls, or in the case samples.
 - 4.2.4.2.2. Analysis, interpretation, and minimum thresholds for acceptance of data. Examples of data quality indicators include PHRED scores, signal intensities or peak heights.

- 4.2.4.3 *Guideline:* Laboratories that work with degraded or low copy number DNA should have an SOP specifically addressing analysis of such samples and subsequent data interpretation.

4.3 Sequencing Standards and Guidelines

- 4.3.1 *Standard:* Laboratories shall have SOPs to address the following:
 - 4.3.1.1 Nucleotide sequence editing and comparison
 - 4.3.1.2 Sequence contamination or mixtures
 - 4.3.1.3 Heteroplasmy
- 4.3.2 *Standard:* Taxonomic identification based on sequence data shall include considerations of:
 - 4.3.2.1 The appropriateness of the reference data, including suitable representation of closely related species
 - 4.3.2.2 Distribution of genetic distances among closest relatives
 - 4.3.2.3 The organism's biogeography, life history and taxonomy
 - 4.3.2.4 Published phylogenies
- 4.3.3 *Standard:* When sequences from public databases (e.g., the National Center for Biotechnology Information's GenBank) are used, analysts shall be aware of the variability in data quality in such databases and make efforts to evaluate its reliability for the taxa under examination.
- 4.3.4 *Guideline:* An identification should not rest on a single sequence from a public database. In the rare instance where additional data are unavailable, limitations of the conclusion should be stated in the report.
- 4.3.5 *Standard:* Statistical estimates of mitochondrial haplotype frequency shall consider the appropriateness and completeness of the reference data.

4.4 STR Standards and Guidelines

- 4.4.1 *Standard:* Laboratories shall have SOPs to address the following:
 - 4.4.1.1 Defining a threshold of signal intensity for alleles used to assign genotypes. These signal intensity criteria are determined by generally accepted values based on the collection platform or are determined empirically by internal validation.
 - 4.4.1.2 Defining a set of minimum criteria for allele designation and genotypes to be included in the final report.
 - 4.4.1.3 Defining bin designation for alleles.
 - 4.4.1.4 Distinguishing artifacts, such as stutter peaks and pull-up peaks, from true allele peaks.
 - 4.4.1.5 Distinguishing between single source, multiple source and partial profile genotypes.

- 4.4.1.6 Use of established formulae (e.g., NRC, 1996) to calculate individualization probability.
- 4.4.1.7 Population assignment, including the use of appropriate statistical support.
- 4.4.2 *Standard:* An internal size standard shall be run with samples to normalize peak migration differences. The sample allele designation shall only be used if the largest and smallest alleles for that sample fall within the range covered by the internal size standard.
- 4.4.3 *Standard:* When data are shared between laboratories, allele calls shall be harmonized (e.g., by the use of quality control samples of known genotype).
- 4.4.4 *Standard:* Each laboratory shall use internally validated panels of loci.
- 4.4.5 *Standard:* All estimates of individualization probabilities shall incorporate an adjustment for population structure.
Note: For taxa with limited mobility or species with non-panmictic breeding, relevant estimates of population structure should be acquired. When theta is not known for a particular species, a conservative adjustment shall be incorporated based on data available from taxa expected to have similar population structure.
- 4.4.6 *Standard:* When doing a population assignment, it is essential that the database include representative geographic coverage and sufficient sample size. If an appropriate population cannot be included in the comparison, the conclusions shall reflect that fact.

4.5 SNP Standards and Guidelines

- 4.5.1 *Standard:* Laboratories shall have SOPs to address the following:
 - 4.5.1.1 SNP amplification (e.g., real-time PCR, allele-specific PCR)
 - 4.5.1.2 Defining a set of minimum criteria for SNP designation (e.g., clustering with positive controls, minimum peak height). These criteria are determined by generally accepted values based on the collection platform or are determined empirically by internal validation.
 - 4.5.1.3 Distinguishing between single source and multiple source samples.
 - 4.5.1.4 Use of established formulae (e.g., NRC, 1996) to calculate individualization probability.
 - 4.5.1.5 Population assignment, including the use of appropriate statistical support.
- 4.5.2 *Guideline:* Positive controls shall include all possible genotypes for each locus. These could be from samples of known genotype or from artificially generated positive control material.
- 4.5.3 *Standard:* Where capillary electrophoresis is being used, an internal size standard shall be run with samples to normalize peak migration differences.

- 4.5.4 *Standard:* When data are shared between laboratories, SNP allele calls shall be harmonized (e.g., by the use of quality control samples of known genotype).
- 4.5.5 *Standard:* Each laboratory shall use internally validated panels of loci.
- 4.5.6 *Standard:* All estimates of individualization probabilities shall incorporate an adjustment for population structure.
- Note: For taxa with limited mobility or species with non-panmictic breeding, relevant estimates of population structure should be acquired. When theta is not known for a particular species, a conservative adjustment shall be incorporated based on data available from taxa expected to have similar population structure.*
- 4.5.7 *Standard:* When doing a population assignment, it is essential that the database include representative geographic coverage and sufficient sample size. If an appropriate population cannot be included in the comparison, the conclusions shall reflect that fact.

5.0 Morphology Standards and Guidelines

Morphology is the study of form. The method of morphological comparison is the basis for classic studies of biological structure and evolution, and is essential in the scientific work of taxonomists, anatomists, paleontologists, and archaeologists, as well as forensic anthropologists. An extensive body of peer-reviewed literature exists that establishes the scientific rigor and utility of morphological comparison techniques.

In a wildlife forensic context, it is the discipline using morphological comparison to identify wildlife parts and products, typically to the family, genus, or species source. Depending on the nature of the evidence, a variety of macroscopic and microscopic comparison techniques may be employed.

It is essential to recognize that almost all analyses performed by a forensic wildlife morphologist are based on class characters, not individual characters. Shared quantitative and/or qualitative morphological characteristics are used by scientists to specify, or define, taxonomic groups, such as families, genera, and species. These class characters are reliably associated with evolutionary lineages down to the species level. Individualization, in contrast, requires the recognition of characters uniquely identifying a particular individual. Individualization based on morphological characters is rarely conducted in wildlife cases.

5.1 General Morphology Standards and Guidelines

5.1.1 Bases for Morphological Determinations

5.1.1.1 *Standard:* The analyst shall examine, interpret, and document morphological similarities between the evidence item and specimens of known species source and/or appropriate scientific reference material.

5.1.1.2 *Guideline:* Scientific references should be used in morphological examinations, as appropriate. Such references may include primary scientific literature, taxonomic monographs, morphometric datasets, identification keys, field guides, and reliable image databases.

5.1.1.3 *Standard:* The analyst shall consider the diagnostic value and inter- and intraspecific variability of the characters being analyzed.

5.1.1.4 *Guideline:* If a species' geographical origin is of particular importance in the interpretation of morphological characters, the most relevant reference materials should be selected.

5.1.1.5 *Guideline:* Analytical documentation and data interpretation in morphology should follow the hierarchy of taxonomy, with characteristics of the order noted first, followed by family-specific characters, and finally those diagnostic to particular genera and species, where possible.

5.1.2 Process of Morphological Examination – External Remains

5.1.2.1 *Standard:* The analyst shall consider the completeness and condition of the evidence, and the presence/absence of taxonomically informative characters.

- 5.1.2.2 *Standard:* When the evidence item does not represent a complete organism, the analyst shall evaluate the appropriate taxonomic level to which identification can be made.
- 5.1.2.3 *Standard:* Age and sex characters of the evidence shall be evaluated, and the analyst shall determine whether available reference materials are appropriate for correct data interpretation and species identification. For example, a morphometric dataset based on adult mammals is usually not useful to identify remains of a juvenile individual.
- 5.1.3 Process of Morphological Examination – Osteological Remains
 - 5.1.3.1 *Standard:* Skeletonization shall not be undertaken without consulting the pertinent party.
 - 5.1.3.2 *Guideline:* Laboratories should have in place an SOP covering any required cleaning of skeletal evidence.
 - 5.1.3.3 *Standard:* Evidence analysis shall include a description of the osteological elements examined, their physical condition, and any taphonomic or anthropogenic alterations.
 - 5.1.3.4 *Guideline:* To determine relative age (adult, subadult, juvenile, or neonate), the analyst should first assess if sufficient material is available for analysis, then assess relevant calibrated characters for the taxon in question (e.g., epiphyseal fusion of skeletal elements or relative completeness of dental eruption or wear in mammals).
- 5.1.4 Process of Morphological Examination – Microscopic Structures
 - 5.1.4.1 *Standard:* Where detailed examination of integumentary structures (such as hair and feathers) is required, macroscopic examinations shall document gross features such as color, pattern, size, or shape, while microscopic examination shall document details of external and/or internal structures.
 - 5.1.4.2 *Standard:* Identifications shall be made with reference to collections of specimens of known taxonomic source (e.g., mounted hairs or feather barbs), or, if not available, to scientific references as defined in Section 5.1.1.2, above.
 - 5.1.4.3 *Guideline:* If microscopic characteristics are examined or compared, evidentiary and reference hairs/feathers/scales should be mounted on glass slides in mounting media of a refractive index close to that of keratin (e.g., xylenes or xylene substitute).
 - 5.1.4.4 *Guideline:* When morphological evidence consists of mammal hair, taxonomic identification should be determined using informative hairs, typically guard hairs.
- 5.1.5 Process of Morphological Examination – Botany
 - 5.1.5.1 *Standard:* Identifications shall be made with reference to collections (e.g., herbariums, xylariums, etc.) of specimens of known taxonomic source or, if not available, to scientific references as defined in Section 5.1.1.2 above.

5.2 **Documentation Standards and Guidelines**

5.2.1 *Standard:* In making a taxonomic identification based on morphological characters, the analyst shall document the following in the case file:

- 5.2.1.1 Type of material received as evidence (e.g., whole or partial organism, bone, tooth, feather, hair, ivory carving, leather, log, disc, veneer, crafted item, etc.).
- 5.2.1.2 Intactness and condition of the evidence.
- 5.2.1.3 Morphological characters used to make the identification.
- 5.2.1.4 Other characters used to aid the identification if used (e.g., wood density of the sample, color etc.).
- 5.2.1.5 Reference materials and/or data sources used to verify identification.

6.0 Chemical Analysis for Taxonomic Identification Standards and Guidelines

Chemical analyses can assist in taxonomic identification of evidence items that cannot be identified by morphological or genetic analyses alone. For example, trees and other plants synthesize phytochemical compounds that are often a distinctive feature of a species or higher taxonomic group. These phytochemicals can be characterized using chemical instruments such as infra-red spectrosopes and mass spectrometers. Similarly, keratin molecules from different species sources can be characterized chemically, providing taxonomic discrimination unobtainable by other techniques.

6.1 General Standards and Guidelines for Chemical Analyses for Taxonomic Identification

- 6.1.1 *Standard:* The analyst shall examine, interpret, and document chemical profile similarities between evidence items and reference materials.
- 6.1.2 *Standard:* The analyst shall consider the diagnostic value of key molecules and inter- and intraspecific variability of the characters being analyzed.
- 6.1.3 *Guideline:* Scientific references used in chemical analyses shall include primary scientific literature and/or taxonomic monographs.
- 6.1.4 *Guideline:* Reference material used to verify identifications should be traceable to a curated collection.
- 6.1.5 *Standard:* Identification that relies on data from a public database should not be based on a single chemical profile, single chemical spectrum or compound. In the rare instance where additional data are unavailable, limitations of the conclusion should be stated in the report.
- 6.1.6 *Standard:* If a species' geographic origin is the analytical question, analysis shall only be attempted if relevant reference materials are available.
- 6.1.7 *Standard:* Taxonomic identification based on chemical fingerprint data shall include considerations of:
 - 6.1.7.1 The appropriateness and completeness of the reference material, including suitable representation of closely related species and look-alikes.
 - 6.1.7.2 The organism's biogeography, life history and taxonomy
 - 6.1.7.3 Relevant published phylogenies

Appendix- References

The references listed here include the key materials upon which these standards and guidelines are based, and some additional references for context or specific issues covered. This is not intended to be an exhaustive list of relevant literature.

References for general section

- Huffman, J. E., & Wallace, J. R. (Eds.). (2012). *Wildlife Forensics: Methods and Applications*. Chichester, UK: Wiley-Blackwell.
- International Laboratory Accreditation Committee. (2014). *ILAC Guide 19: Modules in a Forensic Science Process*. Retrieved from https://ilac.org/latest_ilac_news/ilac-g19082014-published/
- International Organization for Standardization. (2017). *ISO/IEC 17025:2017 General Requirements for the Competence of Testing and Calibration Laboratories*.
- Ogden, R. (2010). Forensic science, genetics and wildlife biology: Getting the right mix for a wildlife DNA forensics lab. *Forensic Science, Medicine, and Pathology*, 6(3), 172–179. <https://doi.org/10.1007/s12024-010-9178-5>
- OSAC Wildlife Forensic Subcommittee Standards. (2014-present). Retrieved August 13, 2018, from <https://www.nist.gov/topics/forensic-science/wildlife-forensics-subcommittee>.
- UNEP-WCMC, & CITES Secretariat. CITES Species+. Retrieved August 9, 2018, from <https://speciesplus.net/species>

Additional References for DNA section

- Cavers, S., Degen, B., Caron, H., Lemes, M. R., Margis, R., Salgueiro, F., & Lowe, A. J. (2005). Optimal sampling strategy for estimation of spatial genetic structure in tree populations. *Heredity*, 95(4), 281–289. <https://doi.org/10.1038/sj.hdy.6800709>
- Dawnay, N., Ogden, R., Thorpe, R. S., Pope, L. C., Dawson, D. A., & McEwing, R. (2008). A forensic STR profiling system for the Eurasian badger: a framework for developing profiling systems for wildlife species. *Forensic Science International: Genetics*, 2(1), 47–53. <https://doi.org/10.1016/j.fsigen.2007.08.006>
- Degen, B., Ward, S. E., Lemes, M. R., Navarro, C., Cavers, S., & Sebbenn, A. M. (2013). Verifying the geographic origin of mahogany (*Swietenia macrophylla* King) with DNA-fingerprints. *Forensic Science International: Genetics*, 7(1), 55–62. <https://doi.org/10.1016/J.FSIGEN.2012.06.003>
- ENFSI APST. (2015). *Best Practice Manual for the Application of Molecular Methods for the Forensic Examination of Non-Human Biological Traces*. Retrieved from <http://enfsi.eu/documents/best-practice-manuals/>
- Ewart, K. M., Frankham, G. J., Mcewing, R., Webster, L. M. I., Ciavaglia, S. A., Linacre, A. M. T., ... Johnson, R. N. (2018). An internationally standardized species identification test for use on suspected seized rhinoceros horn in the illegal wildlife trade. *Forensic Science International: Genetics*, 32, 33–39. <https://doi.org/10.1016/j.fsigen.2017.10.003>
- Federal Bureau of Investigation. (2011). *Quality Assurance Standards for forensic DNA testing laboratories*. Retrieved from <https://www.fbi.gov/file-repository/quality-assurance-standards-for-forensic-dna-testing-laboratories.pdf/view>
- Harris, D. J. (2003). Can you bank on GenBank? *Trends in Ecology & Evolution*, 18(7), 317–

319. [https://doi.org/10.1016/S0169-5347\(03\)00150-2](https://doi.org/10.1016/S0169-5347(03)00150-2)
- Johnson, R. N., Wilson-Wilde, L., & Linacre, A. (2014). Current and future directions of DNA in wildlife forensic science. *Forensic Science International: Genetics*, 10(1), 1–11. <https://doi.org/10.1016/j.fsigen.2013.12.007>
- Linacre, A., Gusmão, L., Hecht, W., Hellmann, A. P. P., Mayr, W. R. R., Parson, W., ... Morling, N. (2011). ISFG: Recommendations regarding the use of non-human (animal) DNA in forensic genetic investigations. *Forensic Science International: Genetics*, 5(5), 501–505. <https://doi.org/10.1016/j.fsigen.2010.10.017>
- Lowe, A. J., & Cross, H. B. (2011). The application of DNA methods to timber tracking and origin verification. *IAWA Journal*, 32(2), 251–262. <https://doi.org/10.1163/22941932-90000055>
- Moore, M. K., & Kornfield, I. L. (2012). Best Practices in Wildlife Forensic DNA. In *Wildlife Forensics: Methods and Applications* (pp. 201–231). Wiley-Blackwell.
- Stephenson, J. J., Campbell, M. R., Hess, J. E., Kozfkay, C., Matala, A. P., McPhee, M. V., ... Wenburg, J. K. (2009). A centralized model for creating shared, standardized, microsatellite data that simplifies inter-laboratory collaboration. *Conservation Genetics*, 10(4), 1145–1149. <https://doi.org/10.1007/s10592-008-9729-4>
- Weir, B. S., & Cockerham, C. C. (1984). Estimating F-Statistics for the Analysis of Population Structure. *Evolution*, 38(6), 1358. <https://doi.org/10.2307/2408641>

Additional References for Vertebrate Morphology

- Baumel, J. J. (1993). *Nomina anatomica avium : an annotated anatomical dictionary of birds* (2nd ed.). Cambridge, MA: Publications of the Nuttall Ornithological Club, No. 23.
- Dove, C., & Koch, S. (2010). Microscopy of Feathers: A Practical Guide for Forensic Feather Identification. *Journal of American Society of Trace Evidence Examiners*, 1(1), 15–61.
- Handbook of the birds of the world alive. (n.d.). Retrieved from <https://www.hbw.com/>
- Knecht, L. (2012). The use of hair morphology in the identification of mammals. In J. E. Huffman & J. R. Wallace (Eds.), *Wildlife Forensics: Methods and Applications* (pp. 129–142). Chichester, UK: Wiley-Blackwell.
- Martin, D. L. (2012). Identification of Reptile Skin Products Using Scale Morphology. In J. E. Huffman & J. R. Wallace (Eds.), *Wildlife Forensics: Methods and Applications* (1st ed, pp. 161–199). Chichester, UK: John Wiley & Sons, Ltd. <https://doi.org/10.1002/9781119953142.ch10>
- Rose, C. L., Hawks, C. A., & Genoways, H. H. (Eds.). (1995). *Storage of natural history collections: A preventive conservation approach*. New York: Society for the Preservation of Natural History Collections. Retrieved from https://books.google.co.uk/books/about/Storage_of_Natural_History_Collections_A.html?id=qPkTAQAIAAJ&redir_esc=y
- Trail, P. W. (2017). Identifying Bald Versus Golden Eagle Bones: A Primer for Wildlife Biologists and Law Enforcement Officers. *Journal of Fish and Wildlife Management*, 8(2), 596–610. <https://doi.org/10.3996/042017-JFWM-035>
- U.S. Fish and Wildlife Service. (n.d.). The feather atlas. Retrieved August 10, 2018, from <https://www.fws.gov/lab/featheratlas/>

von den Driesch, A. (1976). *A guide to the measurement of animal bones from archaeological sites*. Cambridge MA: Peabody Museum of Archaeology and Ethnology, Harvard University.

Wilson, D. E., & Mittermeier, R. A. (Eds.). (2009). *Handbook of the mammals of the world*. Barcelona: Lynx Edicions. Retrieved from <https://www.lynxeds.com/catalog/hmw>

Additional References for Wood Morphology

Gasson, P. (2011). How precise can wood identification be? Wood anatomy's role in support of the legal timber trade, especially CITES. *IAWA Journal*, 32(2), 137–154. <https://doi.org/10.1163/22941932-90000049>

International Association of Wood Anatomists. (1964). Multilingual glossary of terms used in wood anatomy. The Verlagbuchanstalt Konkordia, Winterthur, Switzerland. Retrieved from https://www.iawa-website.org/uploads/soft/Abstracts/IAWA_glossary.pdf

Koch, G., Richter, H.-G., & Schmitt, U. (2011). Design and application of CITESwoodID Computer-aided identification and description of CITES-protected timbers. *IAWA Journal*, 32(2), 213–220. <https://doi.org/10.1163/22941932-90000052>

Mabberley, D. J. (2017). *Mabberley's Plant-book*. Cambridge University Press. <https://doi.org/10.1017/9781316335581>

Miller, R. A., Wiedenhoft, A., & Ribeyron, M. J. (2002). CITES Identification Guide – Tropical Woods Guide. Retrieved from <http://publications.gc.ca/site/eng/9.819974/publication.html>

Missouri Botanical Garden. Tropicos - scientific names of angiosperms. Retrieved August 10, 2018, from <http://www.tropicos.org/>

Richter, H. G., Grosser, D., Heinz, I., & Gasson, P. E. (2004). IAWA list of microscopic features for softwood identification. *IAWA Journal*, 25(1), 1–70. <https://doi.org/10.1163/22941932-90000349>

The Plant List - a working list of all plant species. (2013). Retrieved August 10, 2018, from <http://www.theplantlist.org/>

Wheeler, E. A. (2011). Inside Wood – A Web resource for hardwood anatomy. *IAWA Journal*, 32(2), 199–211. <https://doi.org/10.1163/22941932-90000051>

Wheeler, E. A., & Baas, P. (1998). Wood Identification -A Review. *IAWA Journal*, 19(3), 241–264. <https://doi.org/10.1163/22941932-90001528>

Wheeler, E. A., Baas, P., & Gasson, P. E. (2004). IAWA List of microscopic features for hardwood identification. *IAWA Journal*, 25(1), 1–70. <https://doi.org/10.1163/22941932-90000349>

Additional References for Wood Chemistry

Cody, R. B., Dane, A. J., Dawson-Andoh, B., Adedipe, E. O., & Nkansah, K. (2012). Rapid classification of White Oak (*Quercus alba*) and Northern Red Oak (*Quercus rubra*) by using pyrolysis direct analysis in real time (DARTTM) and time-of-flight mass spectrometry. *Journal of Analytical and Applied Pyrolysis*, 95, 134–137. <https://doi.org/10.1016/J.JAAP.2012.01.018>

Dormontt, E. E., Boner, M., Braun, B., Breulmann, G., Degen, B., Espinoza, E., ... Lowe, A. J. (2015). Forensic timber identification: It's time to integrate disciplines to combat illegal logging. *Biological Conservation*, 191, 790–798. <https://doi.org/10.1016/J.BIOCON.2015.06.038>

- Espinoza, E. O., Lancaster, C. A., Kreitals, N. M., Hata, M., Cody, R. B., & Blanchette, R. A. (2014). Distinguishing wild from cultivated agarwood (*Aquilaria* spp.) using direct analysis in real time and time of-flight mass spectrometry. *Rapid Communications in Mass Spectrometry*, 28(3), 281–289. <https://doi.org/10.1002/rcm.6779>
- Evans, P. D., Mundo, I. A., Wiemann, M. C., Chavarria, G. D., McClure, P. J., Voin, D., & Espinoza, E. O. (2017). Identification of selected CITES-protected Araucariaceae using DART TOFMS. *IAWA Journal*, 38(2), 266–281. <https://doi.org/10.1163/22941932-20170171>
- Finch, K., Espinoza, E., Jones, F. A., & Cronn, R. (2017). Source identification of western Oregon Douglas-fir wood cores using mass spectrometry and random forest classification. *Applications in Plant Sciences*, 5(5). <https://doi.org/10.3732/apps.1600158>
- Hillis, W. E. (1987). *Heartwood and tree exudates*. Springer-Verlag.
- Pastore, T. C. M., Braga, J. W. B., Coradin, V. T. R., Magalhaes, W. L. E., Okino, E. Y. A., Camargos, J. A. A., ... Davrieux, F. (2011). Near infrared spectroscopy (NIRS) as a potential tool for monitoring trade of similar woods: Discrimination of true mahogany, cedar, andiroba, and curupixá. *Holzforschung*, 65, 73-80.