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Humans are Animals, Too: Critical Commonalities and Differences Between Human and Wildlife Forensic Genetics

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ABSTRACT: Wildlife forensics has recently been recognized among the wide variety of forensic science disciplines. This review compares human and wildlife DNA forensics, which use the same genetic tools, but often for far different purposes. Human forensic genetics almost invariably attempts to identify individual perpetrators involved in a given crime. Wildlife forensics often determines whether a crime has occurred. In addition to techniques familiar in human laboratories, like individual matching with STRs, wildlife analysts may be asked to determine the taxonomic identity, geographic source, or sex of evidence items, or the familial relationships or minimum number of individuals among a group of samples. This review highlights the common questions, legal framework, databases, and similar validation requirements to foster understanding between disciplines. Based on this understanding, human and wildlife DNA practitioners may work together and learn from each other in order to elevate the discipline of forensic genetics.

KEYWORDS: forensic science, wildlife forensics, human forensics, individual matching, taxonomic identification, geographic assignment, nuclear DNA, mitochondrial DNA, short tandem repeats, databases

Wildlife forensics is a microcosm of all (human) forensic sciences, applied to the myriad animal and plant species that are protected and managed under local, state, federal, or international law (1-6) The modern field of forensic sciences dates back to the 1800's, and includes analysis of latent fingerprints, questioned documents, hair morphology, serology, and ballistics. Some wildlife forensics applications use these same classic techniques: fingerprints lifted from traps, feathers, eggs, or elephant tusks (7-9), microscopic examination of bullets retrieved from a carcass (10), or toxicological analysis of remains for the presence of poisons (11, 12). Though the evidence items and the species involved differ between wildlife and human forensic laboratories, the point of many of these classic analyses is the same—to determine who was involved and how and where the crime occurred.

More recently, DNA was first used to solve crimes in the 1980s (13), and represented a huge advancement in forensic science. While less commonly in the news, DNA has also been used to solve crimes involving non-human species (3-5, 14-21). This review will focus on wildlife DNA forensics. Many of the genetic tools will be familiar to the reader, while the context of the testing will differ, meaning the decisions made in the laboratory with regards to analysis are markedly different between wildlife and human forensic genetic laboratories (4, 6, 19-23). This review will offer insight into wildlife forensics, highlighting areas where human and wildlife DNA analysts can learn from one another.

As with human crimes, most wildlife crimes do not require forensic DNA analysis: a deer or fish carcass may be easily identified to species based on its morphology, or a paperwork trail documenting receipt of one species but sale of a different, higher value species may be sufficient. But as illicit goods are traded covertly and wildlife products are stripped of recognizable characteristics during processing to become food items (fish fillets, sausages, steaks), decorative items (bone carvings, fur trim, tortoiseshell), or medicinals (ground bone or horn, dried organs), their biological origin becomes obscured. Once items in trade are no longer easily identifiable, illegal items can be difficult to detect without forensic analysis to assist in identifications.

It is easiest to compare and contrast human DNA forensics with wildlife DNA forensics by looking at the common questions both disciplines are asked to answer, and the techniques used to answer those questions (TABLE 1). The vast majority of human forensic DNA analyses are focused on *who* is the source of the unknown DNA, and who can be included or excluded (who committed the crime, who was the victim, who were the witnesses, etc.) as involved in a

crime that has already been deemed to have occurred. Analysts compare genetic profiles to include or exclude an individual as the potential source of the unknown human DNA, often with the help of an offender/arrestee database established for "perpetrator identity discovery" purposes. Wildlife forensic DNA analysts may also be asked to determine if evidence (blood/hair/tissue on clothing, knives, vehicles etc.) originated from a specific individual (3, 4, 6, 22-24). Most often, though, the initial question is whether or not a crime actually occurred. The most frequent question is "What?" (6, 25). What species of animal was used to make this bone carving, and was it a protected species? What animal(s) became the steaks in this hunter's freezer? Wildlife analysts are also often asked "where?" Was this elk from a closed hunting area? Was this whalebone necklace from a whale in US waters, or was it trafficked across international borders? Was this salmon reared in a hatchery, or was it wild?

In all of these cases, the underlying technology is the same between the disciplines, however, the primary questions and techniques relied upon differ. Nuclear DNA (nDNA) short tandem repeat (STR) analysis is used in human forensics for individual matching, and is common to all laboratories. In contrast, mtDNA is a specialty discipline in human forensics, performed in only a handful of laboratories. In wildlife labs those emphases are flipped: mitochondrial DNA (mtDNA) analysis is common to all laboratories, and is used primarily to determine the species of origin of evidentiary items. In wildlife laboratories, nDNA is more specialized, commonly used for individual matching, determination of the minimum number of animals present, or identification of geographic population of origin.

Aside from the common question asked in each discipline, a key difference between wildlife and human forensics concerns the databases on which conclusions are based. Because all human forensic laboratories seek to answer the same "who" question for one ubiquitous organism, *Homo sapiens*, there is considerable impetus for standardization of loci and quality assurance for database generation and interlaboratory comparison. The large number of laboratories contributing data using standardized loci results in many published data sets and quality-controlled law enforcement databases. This is not the case in wildlife forensics, which is characterized by a few small laboratories, each focused on the species assemblage associated with the jurisdictions they serve. Wildlife DNA laboratories, therefore, commonly generate their own, purpose-built validated reference databases constructed from sequences or genotypes for the species of interest (26). Assembling databases for the myriad wild taxa is difficult because of

both the sheer number of species of interest to law enforcement and the lack of opportunity for representative sample collection for most organisms. For species which are legally hunted, tissue samples may be collected from carcasses at game check-in stations or fishing tournaments, but obtaining samples from endangered species is complicated because the species are both rare and legally protected. Collection and possession of endangered species or their parts require permits, and collection should not further imperil already-vulnerable species. Collection of samples from authenticated specimens, therefore, is usually opportunistic, such as from stranded whales or birds that have been electrocuted by power lines. Collecting samples from species with international distributions presents yet another level of difficulty. Organisms that can be freely traded commercially between countries often require scientific collection permits from the country of origin when used for research. For protected species, the permit requirements are even greater as trade between countries in specimens, tissue samples, and even extracted DNA (27, 28) is restricted by the Convention on the International Trade in Endangered Species of Wild Fauna and Flora (CITES, pronounced "sight-ees"). Moving samples across international borders requires collaborators and permits from both the importing and exporting countries, which can take months or years to arrange (29). The difficulty in legally and ethically collecting and transporting samples from endangered animals or plants means that databases for these species tend to be much smaller than those for game or commercial species which can be legally harvested. An additional concern when constructing databases of wild taxa is that most non-human species are more restricted in their movement by natural features such as rivers, lakes, and mountains as well human-associated development, resulting in haplotype and genotype frequencies that can vary widely between populations of the same species in different geographic areas, demanding broad sampling of populations over a species' range. We will explore the need for centralized, managed databases in more detail later, as it is one of wildlife forensics' biggest challenges (4, 5, 23, 24, 26, 29-31).

Wildlife Laws

As considered here, the term "wildlife" encompasses much of the diversity of animal and plant life on Earth. It includes animals traditionally thought of as wildlife, including everyday species such as white-tailed deer, black bears, or songbirds, to far more exotic and rare animals such as pangolins or black rhinos. Wildlife also includes organisms used as food, like tuna or ginseng; in cultural practices such as seahorses used in traditional Chinese medicine; or for

artistic purposes as with Brazilian rosewood used to make a guitar. Though it is difficult to imagine the family dog as "wildlife," domesticated animals often fall into the purview of wildlife forensics as well. While we include plants in our definition of wildlife, this review will focus on the application of forensics to animal species, because animals are the subject of the vast majority of wildlife forensic analyses. We emphasize, however, the urgent need for increased capacity in forensic plant identification (30). The unifying characteristics of our inclusive definition of wildlife are that the organisms are multicelluar (i.e. not viruses or bacteria), and they are not human.

The laws that prohibit people from causing harm to each other or to their property are very familiar to forensics scientists and the public in general. In contrast, the laws that govern wildlife often view non-human species as a resource for public use, and violations center around the details and extent of that use. It may be legal to kill or collect wildlife depending on factors such as the time of year, geographic area, proper licensing, species, or sex. Wildlife, however, are protected by many laws, examples of which are listed in TABLE 2. The laws discussed here are implemented in the United States (US); however, other countries have similar laws defining when hunting is legal, and which flora and fauna are protected. Violations of these laws can include mislabeling, trafficking, and "take," and may result in casework in US wildlife forensic laboratories. Take is variably defined in these statutes, but all definitions include killing, and most include attempts to harm or harass the protected plant or animal. Some statutes, like the US Endangered Species Act (ESA), also provide for protection of the species' habitat, and take can occur via habitat destruction. Illegal take can also include killing the mother of dependent offspring or killing wastefully (termed "wanton destruction").

On the US federal level, the ESA prohibits unauthorized take of over 1400 animal and 900 plant species that are threatened with extinction (32). Internationally, the CITES treaty prohibits trade between signatory countries in more than 1,000 critically endangered species, and limits trade for an additional 35,000 species (33), any of which could be the subject of wildlife forensic analysis.

MtDNA Sequence Databases

Wildlife forensic laboratories use mitochondrial DNA sequence databases primarily for taxonomic identification, and occasionally for population assignment, exclusions (as in human

mtDNA), or for a conservative estimate of the minimum number of individuals. Human forensics has standardized mtDNA loci (hypervariable regions I and II of the control region) and large, quality-controlled databases accessible by human identity laboratories (26, 34). The standardized locus and single focal species also mean that human laboratories can use an agreed-upon reference sequence [revised Cambridge Reference Sequence (35)] and related community-promulgated haplotype naming rules (36). Though there has been a push by some researchers to standardize sequencing part of the mtDNA cytochrome oxidase I (COI) region as a universal "barcode" for animal species identification (37, 38), laboratories may use different loci even when they work on the same taxa because there is no single "best" locus for species identification across all taxa (19, 26, 39-44). Different taxonomic groups diverge at different rates over evolutionary time (45-48), and many taxonomic groups have had gene rearrangements within the mitochondrial genome, potentially impacting any ability to use common sets of primers between species (49-51). All of this variation leads to preferences for certain loci for given taxa and forensic questions.

Once an appropriate DNA locus is identified and sufficient samples have been collected, sequence databases are often generated in-house. There are a few publicly available taxon-specific sequence databases, such as DNA Surveillance (52) and the US Food and Drug Administration's Regulatory Fish Encyclopedia (53), which are compiled from samples validated by taxonomic experts, but they are uncommon. Sequence data are also shared directly between researchers in the small wildlife forensics community, but it is often necessary to use national and international public databases such as the US National Institutes of Health's National Center for Biotechnology Information GenBank and associated databases (the European Nucleotide Archive and DNA Databank of Japan) and the Barcode of Life Database [BOLD (37, 38)], which contain an enormous amount of genetic information. Most journals require sequence data be submitted to GenBank before publication of a related paper, and Genbank and BOLD regularly synchronize published sequences. But unlike the stringent requirements imposed on labs allowed to upload information to human forensic databases like the United States' National DNA Index System, which require annual audits, examiner qualification, and semi-annual proficiency testing in order to connect to the secure FBI-encrypted CODIS network, any individual or institution may upload information to GenBank or BOLD. BOLD has more stringent requirements for sequence submission than GenBank (37), but contains sequences only

for COI for animals (alternative loci have been chosen for plants and fungi). GenBank, on the other hand, has fewer quality control measures, but contains sequences for many more organisms and DNA regions. Both databases are known to contain sequences that are attributed to the wrong species, either due to misidentification of the original organism, contamination, or a mix-up in labeling during laboratory or data analysis (19, 26, 42, 54) Misidentification can occur because many species require some expertise for proper identification, but sample collection is often performed with little expert confirmation. Therefore, sequences from public databases must be validated before being used for forensic comparisons (19, 23, 42, 54-57). This is commonly done by careful review of the publications associated with the sequences in question to determine if the organisms were expertly identified. Ideally, in-house confirmation of sequences from reference specimens is desired. Phylogenetic analysis that includes authenticated in-house sequences and publicly-available sequences from multiple contributing laboratories can also reveal erroneously labeled sequences as outliers (see FIG 1) (42, 54, 62).

STR Databases

Even though thousands of STRs exist in the human genome, only 20 "core" STR loci (along with amelogenin) have been selected for routine use in the US (63, 64), many of which are also used in crime laboratories around the world. Standardization of loci has enabled commercial development of robust, easy-to-use, well-validated kits with allelic ladders for human STR analysis. As with human mtDNA, standardization has allowed development of population-specific allele frequency tables and shared quality-controlled databases. Human laboratories can search evidentiary profiles against those uploaded by other laboratories from crime scenes, convicted offenders, and missing persons, and calculate statistical support for matches.

In wildlife forensics, since few laboratories perform STR analyses for the same species, there is only rare incentive or support to develop standardized loci, kits, and associated databases. While STR markers have been developed on thousands of non-human species for research, there are likely only dozens of taxa for which these markers have been validated and analyzed for forensic purposes [e.g. (65-70)]. A few domesticated taxa have had core loci proposed [e.g. (67, 71-79)]; among those, only horses (*Equus caballus*), cows (*Bos taurus*), and dogs (*Canis lupus familiaris*) have commercial kits available (Stockmarks™ Canine, Bovine and

Equine kits; Canine ISAG STR Parentage kit; Canine Genotypes Panel 1.1 and 2.1; and Bovine Genotypes Panel 1.2 kits; ThermoFisher Scientific, Waltham, MA). None of these kits provide allelic ladders, relying instead on inter-laboratory sample sharing or International Society for Animal Genetics comparison testing when allele calls need to be compared between laboratories (80).

While commercial kits are non-existent for wild taxa, Chinook salmon (*Oncorhynchus tshawytscha*), an important cultural and economic resource in the US Pacific Northwest, stands out as possibly the first wild species for which STR loci were standardized between multiple research laboratories and allelic ladders were developed; the resultant shared database has been validated and used for forensic casework involving ESA-listed populations (68). STR panels and accompanying allelic ladders and databases have also recently been developed and validated to forensic standards for at least two other species, the carpet python [*Morelia spilota,* (69)] and the hen harrier [*Circus cyaneus*, a bird of prey (70)]. Such well-developed STR panels for wild taxa, however, remain the exception rather than the rule.

As with mtDNA, most wildlife laboratories produce their own STR databases for the species they expect in casework. Primers for STR panels are either gleaned from the academic literature or developed in-house. If published primers are used, the laboratory can work with the authors to standardize allele calls to the published data, though often the database will need to be augmented with additional samples to include populations of interest. With panels developed in-house, the laboratory will construct a database using known individuals of the target species from throughout the geographic area of interest to characterize population structure. *Homo sapiens* are a highly vagile species. Despite their mobility and admixture, some human populations still exhibit structure along ethnic lines, which is a reflection of past and current geographic isolation, migration, and culture (81, 82). Allele frequencies in animal (and plant) populations are also shaped by shared evolutionary histories, but populations are likely to exhibit more geographic structure (19, 66). This higher level of population structure leads to labs developing databases for the taxa and geographic regions that they work with. Population structure can be ascertained using a Θ (Fst) value (83, 84). Populations with Fst values of 0.00-0.05 have little to no variation, 0.05-0.15 moderate variation, 0.15-0.25 great variation, and greater than 0.25 very great differentiation. TABLE 3 compares Θ values for various wildlife species to humans, illustrating a wide range of Θ values (66, 85-90).

As with human forensics, STR (and Single Nucleotide Polymorphism, or SNP) population databases are used for statistical support of DNA matching, but they are also used to determine what geographic area an individual is from (called population assignment or geographic assignment) and whether the animal is captive-bred or wild. This type of discrimination is so important to the study of wildlife populations that multiple statistical packages have been developed specifically for that application or incorporate it as part of a larger population genetics analysis package (91-95).

Methods and Applications

Taxonomic Identification

The most common request of wildlife forensic laboratories is taxonomic identification: differentiating between the species protected or managed through jurisdiction-specific laws and the myriad unprotected species (4, 5, 19, 21-23, 25). Taxonomy is the science of classification and naming of organisms based on a system first consistently used by Carl Linnaeus (96) and later standardized internationally (97). Organisms are grouped into taxonomic levels (e.g. family, genus, species) based on shared morphological, behavioral, and/or genetic characteristics as proxies for a shared evolutionary history.

Human forensics must occasionally determine the taxonomic origin of trace evidence from plant or animal material found in casework, but the main focus is usually on a single species, *Homo sapiens*. In wildlife forensics, if an evidence item retains enough morphological characters for an expert to render an identification, morphology is the preferred technique, as it is not only faster and cheaper than other methods, it is considered the "gold standard" for taxonomic identification. Species have always been described based on morphology, though other characteristics such as geographic distribution, behavior, etc. may also be included in the species description. Sequence data (usually mtDNA) are widely used in the discovery and study of new taxa, but have not superseded morphology. Morphological traits are the phenotypic expression of many genes, whereas a classification based on a single-locus sequence may not paint as full a picture (39-41). However, when an evidence item is stripped of its useful morphological features, a wildlife genetic analyst is often called to identify it.

Because of its low discriminatory power for identifying individuals, human forensic scientists use mtDNA primarily as a tool for exclusion, in cases where samples are too degraded to yield nDNA profiles, or with missing persons or unidentified human remains. In contrast, because taxonomic identification is more often sought in wildlife cases, mtDNA analyses are widely used in wildlife forensic labs as a first-line test, and are often the only tests performed. MtDNA is multi-copy, resistant to degradation (98), and evolves quickly enough to resolve differences between most species, but offers conserved regions for primer binding.

Once a wildlife analyst amplifies and sequences DNA from the evidence in question, the sequence is compared iteratively to one or more databases (see FIG 2 for a flow chart illustrating the major steps and decision points in taxonomic identification). Often, a presumptive identification is first obtained by searching the evidence sequence against GenBank using the Basic Local Alignment Search Tool (BLAST) to compare the evidence sequence to the millions of sequences submitted by researchers worldwide (COI "barcode" region sequences can be similarly searched against BOLD). Public database search results are used to guide further data analysis—to see if the sequenced gene region has enough resolution for the taxonomic group, and select the correct in-house database. This basically serves as a check to see if the analyst is on the right track, or needs to change course. For example, if an unknown sample, amplified and sequenced using universal cytochrome *b* primers, matches sequences from a green sea turtle (*Chelonia mydas*) in GenBank, the analyst would then know to compare the sequence to the in-house database for sea turtles (Subfamily Chelonioidea). In the National Oceanic and Atmospheric Administration (NOAA) Northwest Fisheries Science Center (NWFSC) Forensic Laboratory, the in-house database for sea turtles is based on cytochrome *b*, so data analysis could proceed immediately. A different laboratory may have a sea turtle database constructed of COI sequences, which in this example would mean that, after a BLAST search, the analyst would amplify and sequence evidentiary DNA at COI before continuing with data analysis.

For either mtDNA region, data analysis commonly would proceed with aligning the evidence sequence(s) with a database containing sea turtle reference sequences. Ideally, the database would have complete taxon sampling—meaning it has authenticated sequences from all closely related species, each represented by individuals from across their geographic range, to capture the maximum within- and between-species diversity. For taxa like sea turtles, which are well-characterized in the literature and have few species (there are only seven), diagnosing

species is fairly straightforward. Data analysis might involve building a phylogram, or "family tree" to see which species cluster the unknown sequence falls into (See FIG 1 for an example tree), and calculating the proportion of matching bases (known as genetic distance) between the evidence sequence and the nearest database sequence(s). This data would be considered in the context of the published literature, the completeness of the database, and the life history characteristics of the purported species before the analyst would render a conclusion. It should be emphasized that taxonomic identification is a classification, and not an attempt at individualization. Therefore, it is not appropriate for taxonomic identification analyses to produce likelihood ratios or other statistics describing surety of species "matches" (99).

It is worth noting that it is not always necessary to determine the species of origin of evidence samples, as it can be useful (and is sometimes only possible) to establish identity at higher taxonomic levels. For instance, all sawfishes (seven species of shark-like rays in the family Pristidae), which are critically endangered, are CITES- and ESA-listed, so identification of evidence as belonging to this family would support allegation of a violation. All marine mammals, spanning five orders within the class Mammalia, are protected by the marine mammal protection act (MMPA), with a subset of species also protected by the ESA. Since all marine mammals are protected, identifying the evidence to one of these orders supports an MMPA charge, but a species-level diagnosis would be needed to support an ESA charge. The complexity of the laws often leads wildlife forensic analysts to specialize in certain taxa, not only because of the biological knowledge and reference samples needed for their work, but also because of the need to know the legal considerations applicable to those species.

In cases where there is a well-defined investigational question (i.e. a designated set of well-characterized species), an assay using probes, primers, or restriction enzymes to target SNPs can be a fast and low-cost alternative or precursor to sequencing [e.g (100-108)]. These assays are usually designed to detect the target species without a sequencer, which may be beyond the budget of laboratories in developing-world countries that are often the source of organisms targeted by the illegal wildlife trade. Because it is possible for novel, unforseen species to produce a signal that is similar to, but not homologous with, that of a target taxon, these tests are often considered presumptive. Ogden et al. (5) review such tests, and make the distinction that they are methods of *detection*, not identification. These markers, however, can

function well as screening tool to triage samples for sequencing, and are particularly suited for situations where sequencing commonly fails, as when species mixtures are expected [e.g. common food meats (109) or traditional Chinese medicine (105)]. Such assays are best suited to situations where there is a limited number of expected species; they also require more extensive validation than sequencing to ensure the specificity of results (5).

Individual Matching

STRs are used similarly in human and wildlife forensics for matching known and questioned evidence, such as matching blood on a knife in a suspect's truck to a gut pile discovered in an area where hunting is prohibited, or matching tusks from individual elephants across separate shipments to link them to a single trafficker (110).

In scenarios that are likely familiar to human DNA analysts, identifying an individual animal with STRs can be useful in solving human crimes. Possibly the most famous example of using animal DNA to solve a human crime is that of Snowball the cat *(Felis catus*), whose hairs were found on a jacket along with the blood of murder victim Shirley Duguay (111). Stephen O'Brien, a geneticist with the US National Cancer Institute and an expert in feline DNA, worked with a team to validate STR loci, constructed a database from pet cats in the murder area (17), and then used STR analysis to link the hairs found on the jacket to Snowball (111). In this instance, Snowball was not the victim of the crime, but unwittingly provided crucial evidence. Evidence from dogs and other companion animals can similarly help link victim, perpetrator, and crime scene in cases that can require both human and wildlife expertise.

More commonly in wildlife forensic laboratories, scientists must determine if blood, hair, tissue, or other evidentiary items came from a specific individual in order to establish whether the animal was legally taken. Legal take is not a question in the realm of human forensics—it is illegal to kill or assault people no matter what time of year it is or where they are. Even when game animals are taken with a proper license and in-season, it is illegal to waste usable portions of an animal or to kill an animal only for "trophy parts." Therefore, if a wildlife law enforcement officer finds a headless carcass abandoned in a field, evidence collected from a suspect's clothes, vehicle, weapons, gear, and/or home can be used to link the biological material from the evidence to the carcass and the trophy head mounted on the hunter's wall.

Minimum Number of Individuals

STRs are also used to determine the minimum number of individuals/contributors present in submitted evidence (112). In human forensics, the number of contributors is primarily of importance in calculation of statistical weight for mixed profiles. In wildlife, however, it can be important to determine the number of animals represented in a group of evidence items in order to inform charging decisions. In these cases, there's no need to match a known and questioned sample; however, knowing whether the evidence represents more than one individual may be important. For instance, when there are many Chinook salmon fillets or elk (*Cervus canadensis*) steaks in a suspect's freezer, each new illegally-taken individual can represent a "count" of a violation—e.g. an additional ESA violation, or an over-the-bag-limit violation for species that can be legally harvested. It is possible to obtain a conservative estimate of the minimum number of animals represented in evidentiary items using mtDNA haplotypes, but for more precise enumeration, STRs are usually required.

Parentage/Relatedness

Determining parentage is an extremely common request of human DNA laboratories, often to determine a child's biological father (e.g. product of rape analysis), or both biological parents in deceased infants and newborn/stillborn abandonments. While human testing laboratories see thousands of such cases, the question of relatedness is uncommon in wildlife forensics (though more common in wildlife ecological research laboratories), but the technology used—STRs—is often the same. Again, in wildlife labs, the analysis would be to determine if a take was legal or not. For some species (e.g. mountain lions), it is illegal to harvest a female with young at her side, a violation determined through parentage tests. Such analysis also requires a database specific to the species of interest and understanding of the species' natural history as it relates to mating strategies. Parentage and relatedness can also be also useful in determining if captive individuals (farmed) have been taken from the wild or distinguishing between wild and captive-bred individuals (113). This will be discussed in more detail below, with geographic assignment.

DNA Mixtures

One of the most difficult, and often controversial, aspects of human forensic DNA testing is interpretation of mixtures (114). As the ability of geneticists to capture and characterize the

most minute amounts of DNA has improved, so has the likelihood of detecting a genetic signal from individuals who have contaminated a crime scene or sample, but who were not relevant to the crime. Wildlife forensic analysts, however, seldom need to interpret mixed profiles. Validation of mixture interpretation would also be prohibitively costly and time-consuming for the suite of species seen by a wildlife laboratory. Dog fighting or dog attack cases are among the few scenarios when mixture deconvolution may be attempted, arising from the need to determine which dog(s) might have been involved, and in what capacity (115, 116). Fortunately, allele frequencies in dogs are well-characterized (67, 76, 117), and there is even a canine database housed at the UC Davis Veterinary Genetics Laboratory containing profiles from dog-fighting pits and confiscated dogs (118).

Sex Determination

Sex identification in human forensics is based on amelogenin or sex chromosome markers included in commercial STR multiplex kits. In the animal kingdom, mechanisms for sex determination vary widely, from the familiar XY system in mammals, to ZW in birds [where the male is ZZ and the female ZW (119, 120)], and on to taxa like crocodilians, where sex is determined environmentally, not genetically (121). This means that in wildlife forensics, the markers used to characterize sex depends on the species of interest. As with humans, portions of the X and (if present) the Y chromosome are amplified in mammalian taxa, but cervids [e.g. elk, mule deer, moose (122)], bears (123), mountain lions [*Puma concolor;* (124)], and pronghorn [*Antilocapra americana;* (125)], for example, each require different sets of primers for sex determination. Fortunately, the sex of animals with environmental sex determination is rarely of enforcement interest.

Unlike human forensics, where the determination of sex may help identify whether the DNA comes from the victim or a suspect, wildlife forensics uses sex identification to again determine if a crime has occurred. With many game animals, hunting laws are specific to the sex of the animal being harvested. Some areas may be open to hunting females or males only. If a suspect fails to maintain evidence of an animal's sex, forensics must make the determination. Depending on the species, some wildlife labs may incorporate sex markers into an STR panel, akin to human forensics. Alternatively, having sex identification as a separate analysis from STRs can be valuable when the only forensic question is what sex the specimen originated from.

Geographic Assignment

The need to assign animal evidence to a specific geographically-circumscribed population of origin will be unfamiliar to the average forensic scientist, as it is not an analysis done in human forensic laboratories. In wildlife forensics, we mean "population" in the biological sense, as in a group of individuals of the same species that live and breed together in a given geographic region, which gives rise to allele or haplotype frequency differences among populations. In a population assignment case, the analyst is attempting to determine if an individual animal came from a specific population in a specific geographic area. Identifying an evidence item's population of origin can inform investigations by elucidating trade routes [e.g. (126, 127)] or can help determine if a violation has occurred.

The ESA allows for plant and vertebrate species to be listed at the population level as threatened or endangered Distinct Population Segments (DPSs) or Evolutionarily Significant Units (ESUs), while other populations of the same species remain unlisted. One area where this has been especially relevant is in the US Pacific Northwest, where populations of multiple species of salmon (*Oncorhynchus* spp.*)* were listed under the ESA in the 1990's. As more genetic research on populations of impacted species has been conducted, more population-specific listings have been enacted. Enforcement agents, however, often could not simply look at a fish to determine if it was a member of a protected group. In such situations, wildlife forensic scientists can be asked, for instance, to differentiate between non-endangered Coho salmon (*Oncorhynchus kisutch*) fillets and those from endangered populations (e.g. Oregon Coast ESU).

Wildlife forensic scientists may also be asked what country an item originated from, either to determine trade routes or to indicate when an item was illegally trafficked across borders (128). Sometimes these questions cannot be answered genetically because the biology of the wild organisms involved is not bound by geopolitical borders; fortunately, though, sometimes geopolitical borders coincide with biogeographic ones.

At the state level, the question is most often whether an animal was harvested from the permitted area because different areas have different times in the year that it is legal to harvest an animal. Assignment of individuals or groups of individuals to a specific population may be accomplished with STR, SNP, or mtDNA sequence data [e.g.(127-135)]. Population assignment is more difficult than identifying either individuals or species (128), as it requires an enormous and comprehensive database representing the range of populations within a species, and relies

heavily on specialized statistical software to assign an individual or group of individuals to a population and give statistical weight to that assignment. Also, as Ogden and Linacre (128) point out, unlike individual matching or species identification, "geographic assignment techniques are specific to both a single species and a defined investigative question," so it is costly for a laboratory to assemble a database and validate an assignment method if the application and interest is not widely shared. Examples of multi-national coordination demonstrate the level of interest and the investment needed to standardize loci and produce shared databases suitable for forensic population assignment in widespread species: a coastwide Chinook salmon STR baseline was developed at the behest of the Pacific Salmon Commission (68), and the European Commission's FishPopTrace Consortium developed SNP panels and databases for traceability of Atlantic cod (*Gadus morhua*), Atlantic herring (*Clupea harengus*), sole (*Solea solea*) and European hake (*Merluccius merluccius*) (136). These efforts were necessary because these five fish species are all commercially important, vulnerable to overfishing and mislabeling, and in the case of Chinook salmon, have endangered and non-endangered populations that co-mingle in the wild.

A subset of population assignment is determination whether an animal or plant is wild or captive-bred. This is definitely not an issue in human forensics, but can be important for commercially-traded wildlife. In some instances, it can be legal to farm some species for commercial sale, but illegal to sell the same species when it is from the wild. For example, in most states it is not legal to sell wild game for profit, but it is legal to sell the same species if the animal was raised on a farm. It is also illegal to domesticate wild animals for farming or ranching, meaning a person cannot capture an animal from the wild and transport that animal to a farm or ranch for breeding. Determining if jerky came from a farmed elk or a wild elk, for instance, can be a valuable tool for wildlife law enforcement officers. As with other population assignments, establishment of whether a sample is wild or farmed can rely in whole or in part on STR or SNP characterizations (113, 137, 138). If the genotypes of the farmed parents are known, or if the animals within the farm are genetically distinct from animals in the wild, then it is fairly simple to match genotypes of offspring back to the parents, but it becomes increasingly complex in species with many possible parents, such as fish in a hatchery (138, 139). When the broodstock (parents) are not known (e.g. farmed in a foreign country and imported) or individuals are taken from the wild and raised to market size (140), genetics can attempt to

answer the question at hand`, but often additional lines of evidence are needed from non-genetic analyses such as stable isotopes (141, 142), lipid chemistry to reveal components of a captive diet (143), and/or drug chemistry to reveal antibiotics and fungicides used in high-intensity farming (144).

Validation of New Loci and Taxa

Developmental and internal validation of test methods is necessary in both human and wildlife forensics. Developmental validation demonstrates accuracy, precision and reproducibility, and is usually performed by those who designed the method. Internal validation is conducted by individual forensic laboratories, and serves as an in-house demonstration of the reliability and limitations of the analysis (145, 146), and should lead to written quality assurance parameters, interpretation guidelines, and analytical procedures. In wildlife forensics, validation commonly spans both developmental and internal, whereas in the human forensic laboratory, validation is mostly internal, as most of the developmental validation is performed by a kit's manufacturer. In the wildlife forensic laboratory, validation includes generation of population data for statistical support for each species analyzed in the laboratory (66). Given the wide variety of taxa routinely encountered in a wildlife forensic DNA laboratory, validation of new tests is required much more frequently than in a human forensics laboratory. Wildlife forensic laboratories often encounter new taxa, and newly-protected or regulated species may require new testing methods. In general, validation techniques are very similar to those of human forensic science, though wildlife presents unique challenges, detailed below [also see (65, 66, 102, 147)]. TABLE 4 highlights the differences between validation in human and wildlife forensics. Validation can be for two different, but related purposes. The most straightforward of these is bringing new loci on board, which can involve elements of developmental and internal validation. The other, which is more common, is bringing new species on board. This latter one may be limited to searching the literature and analyzing new data sets, but may involve collecting and sequencing new organisms and exploring which candidate loci are best fit for the question at hand.

New Loci

If published markers do not exist or are not suitable for forensic use, laboratories must develop and validate their own sex identification markers, STRs, and/or SNPs loci (55, 65, 66, 68-70, 102, 148) for each species of interest.

Wildlife forensic laboratories must determine which loci will work by searching the literature for appropriate loci or designing markers in-house. For these new loci, conditions for use are optimized and tested for robustness and variability. New STR loci may then undergo another round of testing to develop multiplex reactions. Because most wildlife species lack a reference genome, STR and SNP loci are seldom mapped. Loci can be searched against assembled genomes of the closest related species to attempt to determine chromosomal locations, though this does not always return unambiguous results (65, 66). New loci must be tested for conformance to Hardy-Weinberg expectations to demonstrate the markers do not exhibit problems such as linkage disequilibrium or null alleles, and to characterize population structure (65, 66, 69, 70, 149, 150). Additionally, it can be difficult to test heritability, as pedigrees in wild animals are often unknown, and it is difficult to obtain samples from families.

For taxonomic identification, each laboratory often validates several mtDNA primer sets and genes because there is no single locus that works equally well for all species. Universal primer pairs used routinely for mtDNA amplification and sequencing are tested with varying conditions on several likely species to find a range of conditions under which they work well. Protocols are written to reflect the validated range, as different species have different sequences at primer annealing locations, and may require varying reagent concentrations or thermal cycling parameters to produce good PCR products. A factor likely of lesser concern in human forensics is validation of markers to determine cross-reactivity with other species (66, 102, 147); in wildlife forensics, these test should include common species which might be found in conjunction with the evidence (human, companion, and domesticated animals), common substitutes and look-alike species, and close relatives of the target taxon (5, 19).

Unlike human forensic DNA analysis, deviations from protocols are more frequent due to the broad demands of wildlife DNA casework. Any time there is a deviation, the reason why and the actual change are thoroughly documented in the case notes. Deviations most commonly occur because it is impossible to anticipate and test all taxa that will arrive at the laboratory for analysis. Unanticipated taxa may require amplification of new loci or further optimization of existing ones.

New Taxa

Wildlife DNA laboratories not only have to delimit optimal conditions for amplification and detection of new species, but they also have to validate that those loci can adequately resolve the species in question. This is particularly difficult for closely-related taxa which 1) may share mitochondrial haplotypes due to current or past hybridization events [e.g. (151, 152)] or 2) may not have diverged sufficiently from one another for clear distinction between species at a particular locus [e.g. (153)]. In the first case, the maternally-inherited mtDNA alone cannot resolve the species in question, but addition of a nuclear locus can (152). In the second case, if species are not distinct from one another at one locus, they may be at another locus, or by using multiple additional loci, such as nDNA SNPs. Sometimes, it is either not possible or not practicable with current technology to determine the identity of closely related species (FIG **1** offers an example of two poorly-resolved species) or potential hybrids (60, 61); in these instances, wildlife analysts often will only identify the evidence to a higher a taxonomic level, such as genus.

Sometimes new taxa are revealed only when presumptive taxonomic identifications are accomplished via comparison with public databases. Then, a literature search begins in order to determine if the locus was appropriate to resolve the presumptive species in question, or if another locus would be better. In-house reference databases can also be assembled post-hoc using samples available in the laboratory's archive (or obtained specifically for this analysis) in combination with reliable published data.

Examples

Several case examples are included below in order to illustrate the types of cases encountered in wildlife forensics.

Case Example 1: Mule Deer Poaching

If someone is not an avid hunter or does not live in an area with a hunting culture, some of the wildlife forensic cases at the state level may not seem important. The following case example, however, exhibits the importance of hunting regulations to the community. In this particular case, a person who was very well-known in his community poached a mule deer that was well-known for his unique "rack" of antlers.

The laboratory received three items from the submitting law enforcement officer. Two of the items were from kill sites in two separate areas, and one item was a tissue sample from a mule deer. Species identification was performed on the samples from the two kill sites using a variety of serologic and enzymatic tests. In this particular case, a counter immunoelectrophoresis was used to determine the samples originated from the Cervid family, the phosphoglucose isomerase enzyme system was used to determine the samples originated from deer, and a combination of isoelectric focusing and a western blot was used to determine the samples originated from mule deer. DNA from all three items was amplified using PCR at twelve variable microsatellite loci. The analysis showed that all three items exhibited identical genotypes. Based on a mule deer database for the local population, the likelihood of another mule deer, chosen randomly from the same population, having the same DNA profile as the DNA profile obtained from the evidence was less than one in three million. The analyst thought it highly improbable that two items from two separate kill sites matched the same mule deer. Suspecting that there had been an error, the laboratory reanalyzed all of the evidence, yielding the same results. The analyst then called the law enforcement officer with the results, which is when all the background information was revealed to the laboratory.

As noted, the person charged with the poaching was well-known in the community, and other community members knew the area in which he was licensed to hunt. The mule deer was also very well-known and highly coveted for his unique rack. Community members knew where this deer lived, and it was not the same area that the suspect was licensed to hunt, prompting calls to law enforcement. When the officer interviewed the suspect, he said that he had harvested the deer in the area for which he had a license, and took the officer to see the kill site (FIG 3). The officer noted a lack of blood at the kill site and the presence of juniper leaves when there were no juniper trees in the area, and suspected that something was amiss. He searched for and found another kill site in the area in which the deer usually lived, and which had adjacent juniper trees (FIG 4). The suspect had knowingly harvested the deer in an area for which he did not have a license, and then moved evidence of the kill to the area where he was allowed to hunt. The DNA results were key to backing up the officer's suspicion that the defendant had poached the deer. This case went to a jury trial, and the suspect was found guilty of taking an antlered mule deer in the wrong area. He was sentenced to pay $10,000 in fines, $4,000 in restitution, and $30 in court cost, as well as 180 days in jail (suspended), and loss of his hunting privileges for five years.

The tests used for species identification were validated in-house as were the STR markers used for individual matching. The lab houses its own database consisting of 27,093 samples from various species, approximately 4,355 of which are mule deer.

Case Example 2: Living Off the Land

This case was unique due to the number and types of samples submitted to the WGFD Wildlife Forensic Laboratory. Officers investigating several break-ins of several cabins and trucks discovered a camp tucked away in the woods with animal remains in various stages of decay strewn about (FIG 5 and FIG 6). The Game Warden submitted over one hundred samples, for which the laboratory performed species identification through a combination of DNA sequencing, enzymatic, and serological tests. Once species identities were established, items were subjected to sex determination and STR analysis to determine the minimum number of animals present. Results included one male moose (*Alces alces*), one female moose, one male elk (*Cervus canadensis*), two female mule deer (*Odocoileus hemionus*) and one male mule deer.

The suspect was charged with taking antlered moose, elk, and deer out of season and without licenses, along with some other species. Charges also included taking a bull elk with an illegal firearm, and attempting to take a sandhill crane without a license, in a closed season, with an illegal firearm and illegal shot. The suspect plead guilty to fifteen wildlife violations and was fined $1,760 and assessed $2,250 in restitution for the antlered moose, elk and deer. The suspect was also ordered to serve forty-three days in jail and had his Wyoming hunting and fishing license privileges revoked for 45 years.

Case Example 3: Sea Turtle Poaching

A NOAA National Marine Fisheries Service Office of Law Enforcement Special Agent submitted to the NWFSC Forensic Laboratory tissue samples known to be from a sea turtle head and carapace, a knife, a speargun, a cotton swab with blood on it, and four empty mesh fish bags, commonly used to keep the catch in when spearfishing. All were confiscated from a fisherman's boat in the US Virgin Islands. A second submission from the same agent comprised tissue samples from partial turtle carcasses found on a nearby beach. The agent requested species identification, and "matching" between the tissue sample from the boat and the other items to determine if the carcasses from the second submission were related to the evidence from the first submission. The laboratory had not validated sea turtle STRs for forensic use. The agent was

informed, however, that mtDNA sequencing might be used to determine if more than one individual was represented among the evidence items.

DNA was extracted from all of the items, amplified, and sequenced using the lab's standard cytb-based sea turtle species identification protocol [based on (154)]. The control region was then amplified from items subsequently identified as sea turtle, as it is commonly used in the sea turtle literature for population genetic analyses and population assignment (155-157), and is more variable than cytb and can be more useful for determining the minimum number of individuals present.

The two tissue samples from the boat, the cotton swab, and subsamples from the knife and speargun all yielded identical haplotypes at cytb. To determine species, this haplotype was aligned with in-house reference samples, and a phylogram was constructed with specialized software to visualize its position on the "family tree" of sea turtles (FIG 7). The phylogram showed the unknown haplotype was identical to in-house green turtle (*Chelonia mydas*) reference haplotype Cm1 and closely allied with other green haplotypes, leading to the conclusion that it came from a green turtle. Additional subsamples taken from the speargun and from one fish bag produced two cytb sequences that were not similar to any species in the laboratory's databases. A GenBank search revealed that one of these sequences exhibited low similarity to the family *Sparidae* (porgy family), and the other had high similarity to (95–99%) to bigeye scad (*Selar crumenophthalmus*). As the laboratory did not have databases for these fishes, and they did not likely represent violations, they were reported as "unidentified fish." Three of the fish bags yielded no amplicons, and were not pursued further.

The green turtle samples produced a single control region haplotype, leading to the conclusion that the evidence was from a minimum of one green turtle. To determine how common the haplotype was in the Virgin Islands, it was compared to a green turtle haplotype database maintained by the Archie Carr Center for Sea Turtle Research (158). The closest population in which the haplotype had been recorded was the Bahamas, where it was quite common and therefore not very informative for minimum number of individuals, occurring in 62 of 80 sampled turtles (155).

The two tissue samples from the second submission were analyzed as for the first submission. These yielded one hawksbill turtle cytb haplotype, and two different control region haplotypes, indicating at least two individuals. The combined results led to a conclusion that

minimally two hawksbills and one green turtle were present among the two evidence submissions, and there was no evidence that the hawksbill carcasses were linked to the boat with the green turtle. The fisherman was convicted of possession and transportation of an endangered green turtle and sentenced to 45 days in prison, 5 years of supervised release, and 200 hours of community service (159). In this case, laboratory analysis provided evidence that the defendant had killed a green sea turtle, but did not substantiate the agent's suspicion that the defendant was responsible for two nearby dead turtles.

Case Example 4: Whale Bone Pendant

In 2016, the NWFSC Forensic Laboratory received a carved bone necklace (FIG 8), suspected to be humpback whale (*Megaptera novaeangliae*) or sperm whale (*Physeter macrocephalus*), as part of a joint US Fish and Wildlife Service and NOAA investigation. All sperm whales are considered endangered, but as of 2016, only five of 14 distinct population segments (DPSs) of humpback whales are listed (160, 161). The agents requested that the laboratory determine whether the bone was from a whale, and if so, if that whale was from a listed population. The defendant claimed that the bones originated in Alaska (which would represent a MMPA violation), but the agent suspected the bone to have originated from Fiji or Tonga (which would represent both MMPA and Lacey Act Violations).

A piece of the pendant was pulverized in a freezer mill and DNA extracted from bone powder based on (162), and the extracted DNA was further purified with a silica extraction (163). DNA was amplified and sequenced using marine mammal CR primers (164, 165), yielding a 326 bp haplotype. This haplotype was compared to the laboratory's whale CR database, and shared 98% identity with the nearest humpback whale haplotype. This is not a particularly close match, but is within the known variability of humpback whales at CR, leading the analyst to conclude that the bone originated from a humpback whale

Once the analyst identified the evidence as humpback, the remaining questions centered on the evidence item's population of origin. Humpback whales feed near the Earth's poles in summer, migrating to equatorial breeding grounds in winter. Northern Hemisphere (NH) and Southern Hemisphere (SH) humpback populations do not mix, as they seldom cross the equator, and do not occur near each other at the same time. Humpbacks, especially females, also show fidelity to certain feeding and breeding grounds, leading to strong genetic differences between

ocean basins, particularly in the mitochondrial genome. Less dramatic, but still detectable, differences exist between the 14 DPSs within ocean basins (166).

Whale researchers Dr. C. Scott Baker and Debbie Steel at Oregon State University generously contributed authenticated humpback whale sequences and their corresponding geographic data from global whale datasets (90, 167, 168) for the Laboratory's use. The Laboratory added additional novel published haplotypes with geographic data (169, 170), which were gleaned from GenBank. Comparison of the evidence sequence to the newly-assembled global humpback database revealed that it was identical to a SH haplotype designated SP50 (90), which had been recorded only in the Oceania and Southeastern Pacific DPSs (167, 168). It was also identical to a slightly shorter haplotype, HAN021, which had been recorded in Antarctica (170). The analyst concluded that the whale bone pendant "originated from a Southern Hemisphere haplotype, which has not been recorded in North American or Hawaiian waters." This conclusion ruled out an ESA violation, since all of the endangered and threatened populations are NH populations.

This one pendant was a small part of a much larger case, involving a 2-year undercover investigation and the largest seizure of whale bone in Hawaii history. The defendants, a husband and wife, were convicted of conspiracy, smuggling, and Lacey Act violations, and had to forfeit seized property worth up to approximately $270,000. The couple was also sentenced to six months of home incarceration, five years of probation, and a $40,000 fine (171).

This case involved a new application of an existing method, and illustrates a number of different points. First, in the species identification analysis, the evidence sequence bore only 98% similarity to the nearest humpback in the Laboratory's database, but it was more similar to the humpbacks than it was to all of the other baleen whales. There were only NH humpbacks in the initial database comparison, and because of their life history habits, the NH and SH whales are distinct [summarized in (166)]. The relatively low similarity to the nearest humpback highlighted that the database was missing the whales most similar to the evidence. This incomplete geographic sampling, though solved with knowledge of the literature and later construction of a worldwide humpback database, produced a match that was initially equivocal, a result akin to what would happen in a species identification comparison with incomplete taxon sampling.

As for the geographic question, it was accomplished with a new analysis of the same sequence that was used for species identification. It required collaboration with an expert in

whale genetics, and incorporated sequence data from publications which collectively had 53 authors from around the world (90, 167-170), a testament to the effort required to assemble a comprehensive dataset for geographic assignment of a species with global distribution.

Discussion

Both human and wildlife forensic DNA laboratories use strong science to answer questions along a continuum from a fine (who is it?) to a broad (what is it?) level of resolution. Human laboratories and some wildlife labs concentrate their expertise at the finer end of the spectrum, while other wildlife laboratories concentrate on broader resolution. Because there is so much overlap, there is much that wildlife and human forensic DNA laboratories can learn from each other.

Butler (172) has postulated that there are four major themes and time periods for human forensic DNA analysis: 1) exploration, where different markers were tried, and a need for standardization was realized, 2) stabilization and standardization, where core loci were selected, and kits and national databases were launched, 3) growth, where kits and databases were expanded and rapid DNA was launched, and 4) the current "sophistication" phase, where a core set of standardized tools are honed and expanded, while balancing high caseload and increasingly sensitive methods with privacy concerns. Wildlife laboratories, on the other hand, straddle several of Butler's developmental time periods because of the lack of significant government investment and the (almost) negligible corporate investment to enable standardization of loci and establishment and expansion of centralized databases, as well as variable nature of the work. As wildlife forensics continues to mature, it is unlikely that it will mirror the path of human forensics, because different applications will develop and mature at stages along the way, and though centralized, quality-controlled wildlife databases may come into being for some taxa, others will remain in individual forensic laboratories, which will be centers of specialization.

Wildlife laboratories sit at the crossroads between conservation-focused genetics research, policy, and the law (5, 22), and tend to be closely allied with academic research institutions or with agencies whose primary aim is management of natural resources. As such, wildlife forensics enjoys a strong culture of hypothesis-driven science, and research is commonly

conducted within and between wildlife forensic laboratories and academic partners. Interpretation of results is considered in a larger framework of evolutionary theory and familiarity with the academic literature regarding each species' ecology, biogeography, and population genetics (22). Human forensic DNA scientists could explore the roots of their science and evolutionary perspectives via more interaction with and understanding of wildlife forensics. Wildlife forensics offers an example of bridging the gap between academic research institutions and applied science for forensic individualization and classification (5), which recent authors have urged for the forensic sciences as a whole (114, 173-174).

Though the lack of standardized loci among wildlife laboratories presents an impediment to data sharing and standardization, it also means that the field is not tied to large corporate and government investment in legacy databases which strongly compel new advances to be backwards-compatible (172). This enables wildlife laboratories, while being mindful of the courts' requirements for reliable science, to be relatively early testers and adopters of new technologies. SNPs and genotyping by sequencing (GBS) are beginning to be used in casework in the NWFSC Forensic and other wildlife laboratories, and should obviate the need for allelic ladders for STRs, easing future data sharing efforts.

Today, next-generation sequencing (NGS) technologies are not only the province of wildlife laboratories—NGS is being explored in human forensic laboratories as well. Human forensic laboratories are validating GBS and whole mtDNA sequencing (175) for human identity testing using, estimating ancestry (81) and predicting phenotypes from DNA for investigational purposes (176). In an application that crosses over into wildlife forensics, NGS is also being used for applications such as forensic palynology for geolocation (177). Similarly, wildlife laboratories are exploring NGS for difficult samples such as traditional Chinese medicines (178) and food additives (179), which contain multiple species and are heavily processed, presenting challenges for traditional sequencing techniques. Cross-talk between disciplines on roadblocks and successes in such research could be critical in improving outcomes.

Wildlife laboratories also have much to learn from the "process" side of human forensics. Few wildlife laboratories are accredited, likely because of a lack of staffing, funding, and infrastructure (25). As wildlife laboratories work towards accreditation, however, they can

learn from their human colleagues' lead in quality management, and implement standardization where possible. There are already a number of applicable standards and guidelines for wildlife forensics, [e.g. (55, 180, 181)], and laboratories will soon be able to request a compliance audit against Society for Wildlife Forensic Science (SWFS) standards and guidelines. More wildlife analysts should also undergo certification and regular proficiency testing (both are offered through SWFS) in order to show third-party recognition of the rigor of their work.

Cross pollination between the wildlife and human forensic communities through efforts such as the US National Institute of Standards and Technology's Organization of Scientific Area Committees [OSAC; (182)] and European Network of Forensic Science Institutes (ENFSI) are fostering better understanding of areas that can be standardized between disciplines, and those where such standardization is not applicable. Within the OSAC Biology Scientific Area Committee, subcommittees specializing in human and wildlife forensic genetics have come together under one roof for the first time in the US, allowing experts to explore the similarities and differences between their respective areas of practice. The subcommittees have already found common ground to produce several documents, most of these pertaining to analyst training. For example, the two disciplines have shared documents in forensic DNA analysis training programs specifically addressing serology, DNA isolation and purification methods, quantification, STR typing, sequencing, and mtDNA amplification. In documents where cross-species standardization was not possible, titles for the first time included "human" or "wildlife" to indicate when the application was not intended for *all* forensic DNA analyses. Following the lead of human forensics, the OSAC Wildlife Forensics Subcommittee-affiliated laboratories have started research to identify standardized panels of STRs for those species regularly encountered, and to develop associated allelic ladders.

In the OSAC crucible where we seek to standardize what we can between human and wildlife DNA, both sides continue to learn about the minutia and concerns of the other discipline, and it is our hope that this review spurs further conversations and understanding throughout the forensics community. Wildlife and human DNA forensics are similar in methodology but markedly different in application. Even though the posed questions and routes to the answers may differ, both disciplines can work together to produce the best scientific products possible.

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TABLE 1—*Similarities and differences between the common questions in human and wildlife DNA forensics.*

Common Questions	**DNA Used in Human Cases?**	**DNA Used in Wildlife Cases?**
What species (etc.) is it? (taxonomic identification)	Rarely	Yes; usually mtDNA
Who is it? (Individual matching)	Yes; nDNA	Yes; nDNA, rarely mtDNA
How many individuals are represented?	Yes; nDNA, sometimes mtDNA	Yes; nDNA, mtDNA
What sex is it?	Amelogenin; Y chromosome analysis	Yes; several different markers
Is it wild, or was it captive-bred?	No	Yes; nDNA, mtDNA
Where is it from? (geographic assignment)	No	Yes; nDNA, mtDNA
Are these two individuals	Yes; nDNA	Yes; nDNA

related?		
If there is a mixture of individuals, who are they?	Common; nDNA	Rarely; nDNA

TABLE 2—*Example wildlife protection laws that can result in casework in US forensic laboratories.*

Law/Treaty, (Abbreviation, year first enacted)	**Purpose**
	International agreements
Migratory Bird Convention (n/a, 1916)	Agreement between Canada and the US to protect migratory birds.
Convention on the International Trade in Endangered Species of Wild Fauna and Flora (CITES, 1975)	An international agreement between 182 signatory countries. Tracks authorized trade and prohibits unauthorized trafficking in >35,000 species of animals and plants between signatory nations. As implemented in US law, infractions can result in civil or criminal penalties.
Agreement on Port State Measures to Prevent, Deter and Eliminate Illegal, Unreported and Unregulated	An international treaty currently covering 85 nations, it requires parties to screen foreign-flagged fishing vessels entering ports and verify information about the vessels' identity and cargo. Ports may deny docking and services to

Fishing (PSMA, 2009)	vessels suspected of illegal fishing.
US Federal laws	
Endangered Species Act (ESA, 1973)	Prohibits unauthorized take and trafficking of approximately 2,300 threatened and endangered species and their parts. Also provides for protection of their habitat.
Federal Food, Drug, and Cosmetic Act (FD&C, 1938)	Prohibits false or misleading labeling of food. There are many such regulations covering truth in labeling of seafoods, meats, vegetables and grains.
Lacey Act (n/a, 1900)	Among other things, prohibits trade in wildlife that have been illegally taken, possessed, transported, or sold. Also prohibits making or submitting a false record, account, or label for fish or wildlife that has been or is intended to be transported in interstate or foreign commerce.
Magnuson-Stevens Fishery Conservation and Management Act (MSFCMA, 1976)	Manages fisheries through regional Fishery Management Councils, which set open seasons, catch limits, gear restrictions, and other rules.
Migratory Bird Treaty Act (MBTA, 1918)	US implementation of the Migratory Bird Treaty. Protects over 800 bird species and their parts (eggs, feathers) from take within the US, or trafficking between the US and Canada, Mexico, Japan, Great Britain, and Russia.
Marine Mammal Protection	Prohibits unauthorized take and trafficking of all marine

Act (MMPA, 1972)	mammals and their parts.

US State laws	
The Interstate Wildlife Violator Compact (IWVC, 1989)	Forty-seven member states that share information on violations by sportsman fishing, hunting, and trapping. A suspension or revocation of privileges in one state is recognized by all member states.
Various regulations set by individual states (Statute names and years vary)	Some statutes list endangered species, but most cover common violations related to hunting and fishing: too many animals harvested, wrong sex, wrong species, hunting out of season or in a closed area.

TABLE 3—Example Fst/Θ values calculated from mtDNA sequences or nDNA STRs. WGFD=Wyoming Game and Fish Department unpublished data.

Species	State/Area	Locus	Average pairwise Fst/Θ	Citation
Human, *Homo sapiens*	World	nDNA	0.0038 to 0.1050	(85)
Elk, *Cervus canadensis*	Wyoming	nDNA	0.042 to 0.155	WGFD
Mule Deer, *Odocoileus*	Wyoming	nDNA	0.000 to 0.176	WGFD

hemionus				
Pronghorn, *Antilocapra americana*	Wyoming	nDNA	0.0003 to 0.129	WGFD
Bobcat, *Lynx rufus*	Wyoming	nDNA	-0.014 to 0.712	WGFD
Eurasian Badger, *Meles meles*	United Kingdom	nDNA	0.12	(66)
Savannah Elephant, *Loxodonta africana*	Kenya	mtDNA	-0.033 to 0.654	(86)
		nDNA	-0.008 to 0.047	
Ground pangolin, *Smutsia temminckii*	Southern Africa	mtDNA	0.000 to 0.564	(87)
Tiger, *Panthera tigris*	Nepal	nDNA	0.08 to 0.21	(88)
Chinook salmon life history types, *Oncorhynchus tshawytscha*	Snake River, WA, OR, ID	nDNA	0.080 to 0.120	(89)
Humpback whale, *Megaptera novaeangliae*	Worldwide	mtDNA	0.0858 to 0.1755	(90)

	nDNA	0.0400 to 0.1030

TABLE 4—*Validation comparison*

	Human	**Wildlife**
Number of species of interest	One	Potentially tens of thousands
Commercial kits available for tests	Yes	Rarely
Loci standardized among laboratories	Yes (some markers still deviate)	Rarely
Allelic ladders available	Yes	Very rarely
Quality-controlled databases shared between laboratories	Yes	Rarely for STRs; limited for mtDNA
Reference genome available	Yes	Sometimes
Locations of STRs within genome is mapped	Yes	Rarely
Developmental validation done by laboratory	Rarely	Commonly

Internal validation done by laboratory	Yes	Yes

Figure Legends

FIG. 1—*Phylogram (58) based on 644 base pairs of cytb, showing the relationships between representative genetic haplotypes of western Atlantic snappers, commercially important fish which are commonly misidentified or fraudulently substituted (59). Species names are followed by each haplotype's accession number or laboratory identifier in parentheses. Haplotypes sources are as follows: validated in-house reference sequences (○); sequences from GenBank (◊); and hypothetical unknown sequences (■). GenBank sequences can capture species diversity not represented in the laboratory's collection; in-house reference sequences allow the analyst to check the validity of GenBank sequences. Several things are of interest in this tree: 1) Most of the haplotypes form tight, single-species clusters, but the Northern and Southern Red snapper haplotypes are interleaved. These two nominal species are morphologically very similar and genetically indistinguishable, and may constitute only one species (60, 61). 2) "Unknown fish 1" is closest to, but outside of, the red snapper group. It is actually a sequence from Pacific red snapper (L. peru), a species which is missing from the tree. When a database has incomplete taxon sampling, an unknown sequence will group with the closest-related available taxon, which could lead to an incorrect identification if other information is not properly considered (55). 3) "Unknown fish 2" appears well within the Lane snapper cluster, and would be identified as such in casework based on this placement, genetic distance, and other considerations of the species' biology. 4) The "Lutjanus cyanopterus GBXXXXXX", a hypothetical Cubera snapper sequence downloaded from GenBank, appears in the middle of the Grey snapper cluster. The most likely explanation for this is that it is from a Grey snapper which was mis-identified as Cubera snapper, sequenced, and accessioned to GenBank with the wrong name. An "out of place" species like this would raise red flags to the analyst and require further research; such sequences would not be used as reference sequences unless their source identity could be substantiated.*

FIG. 2—*Flow chart for laboratory and data analysis in taxonomic identification cases.*

FIG. 3—*Kill site in area where suspect was licensed to hunt.*

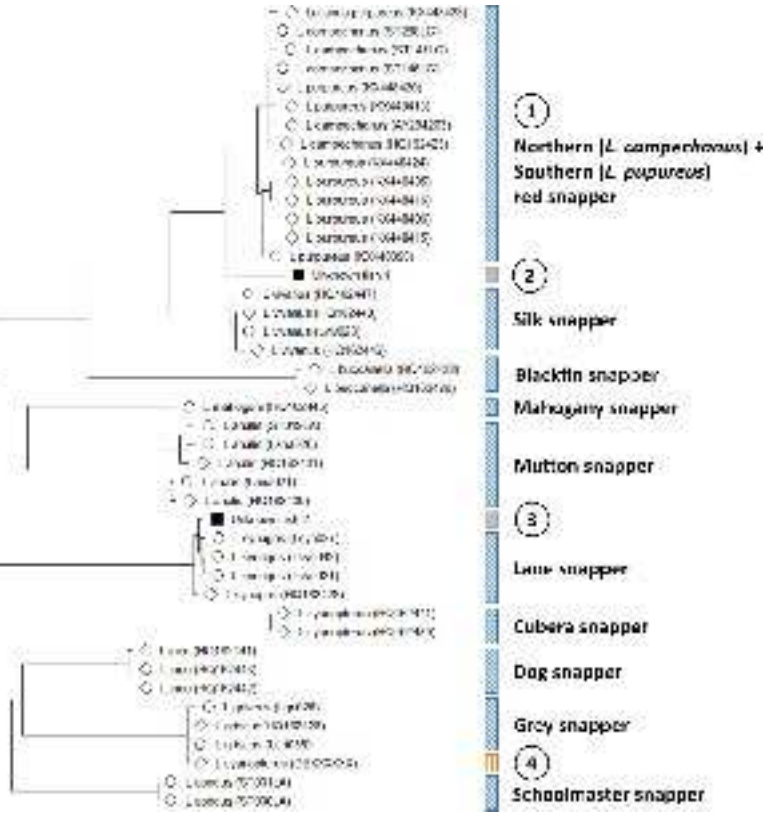
FIG. 4—*Second kill site in area where suspect was not licensed to hunt, and the deer was known to live.*

FIG. 5—*Remains at the campsite.*

FIG. 6—*This is an example of the condition of the evidence at the campsite*.

FIG. 7—*Phylogram (58) based on 400 base pairs of cytb, showing that the evidence sequence haplotypes from the first and second submissions are closest to the green and hawksbill turtle reference haplotypes. Reference haplotypes are designated by the first letter of the genus and species names, followed by a number*.

FIG. 8—*Front (A) and back (B) of a whale bone pendant submitted to the NOAA NWFSC Forensic Laboratory for species and population of origin identification.*



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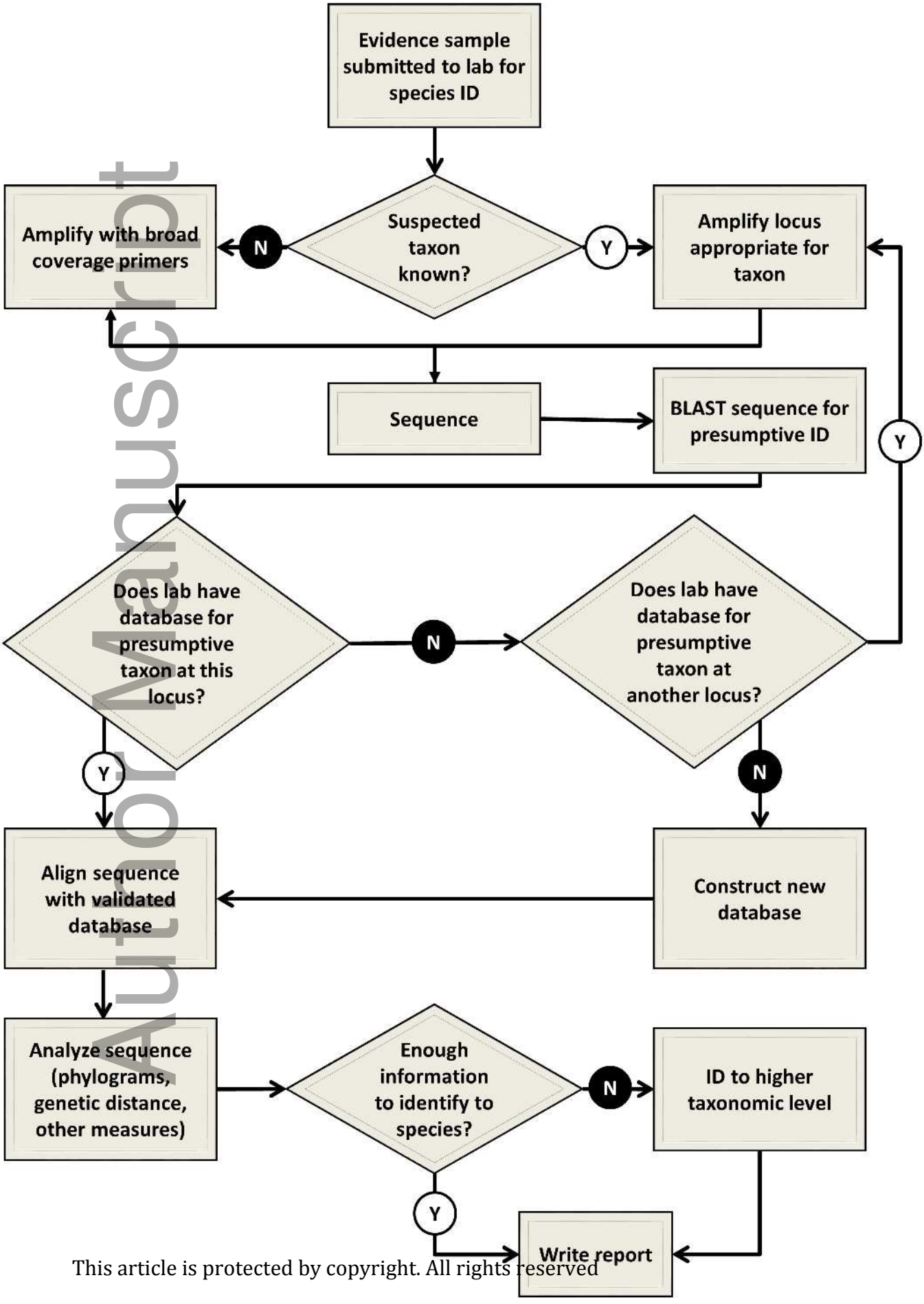

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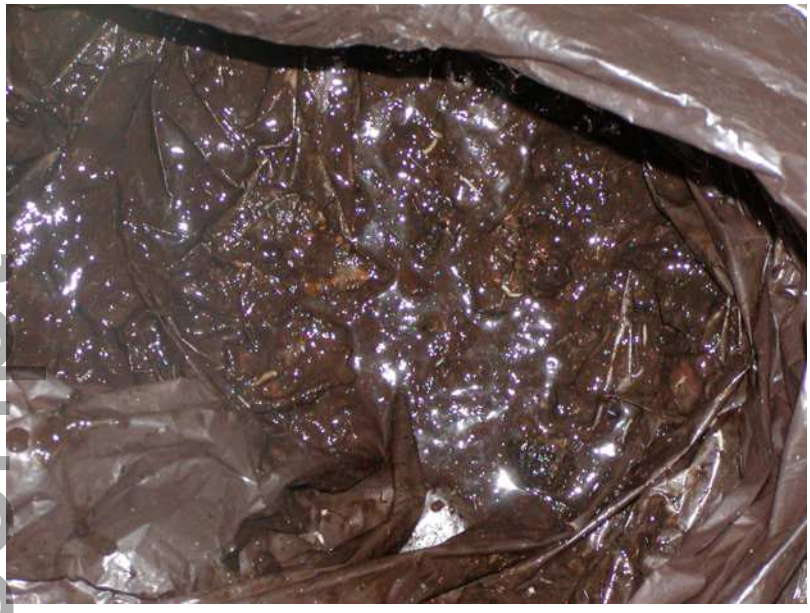

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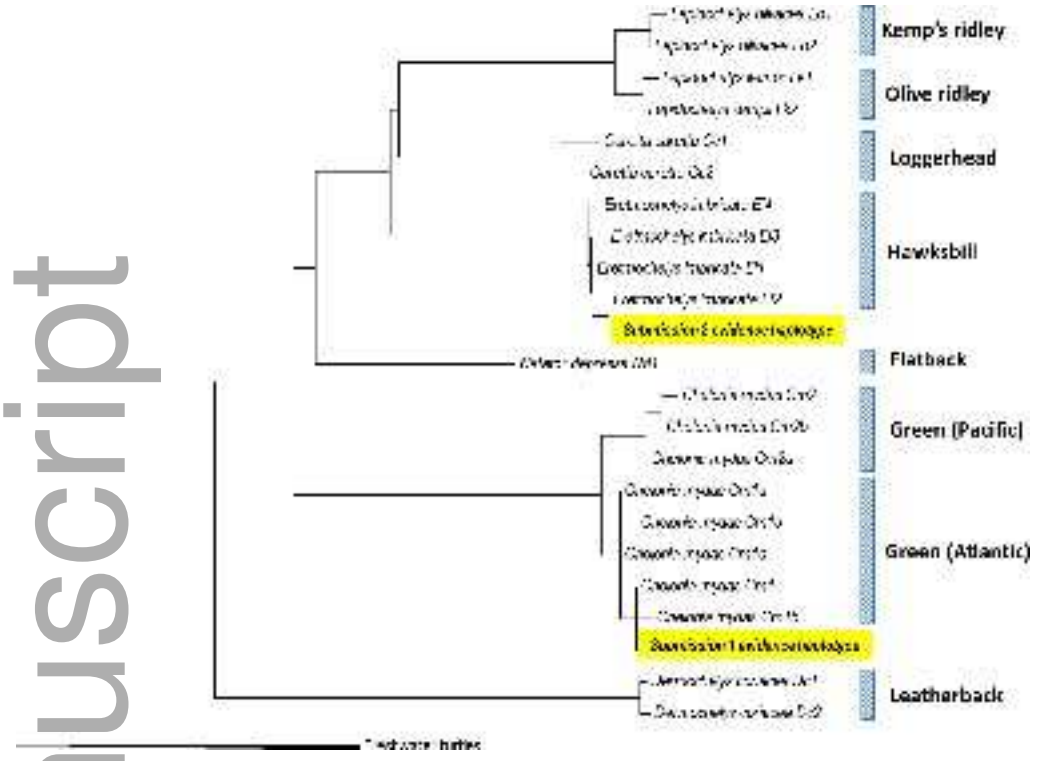

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