DNA Barcoding for the Identification and Authentication of Animal Species in Traditional Medicine

Fan Yang,1,2 Fei Ding,3 Hong Chen,3 Mingqi He,3 Shixin Zhu,3 Xin Ma,1,2 Li Jiang,1,2 and Haifeng Li3

1Institute of Forensic Science, Ministry of Public Security, Beijing 100038, China
2Beijing Engineering Research Center of Crime Scene Evidence Examination, Institute of Forensic Science, Beijing 100038, China
3Center for Bioresources & Drug Discovery and School of Biosciences & Biopharmaceutics, Guangdong Pharmaceutical University, Guangzhou, Guangdong 510006, China

Correspondence should be addressed to Haifeng Li; lihf@gdpu.edu.cn

Received 19 December 2017; Accepted 11 March 2018; Published 22 April 2018

Copyright © 2018 Fan Yang et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Animal-based traditional medicine not only plays a significant role in therapeutic practices worldwide but also provides a potential compound library for drug discovery. However, persistent hunting and illegal trade markedly threaten numerous medicinal animal species, and increasing demand further provokes the emergence of various adulterants. As the conventional methods are difficult and time-consuming to detect processed products or identify animal species with similar morphology, developing novel authentication methods for animal-based traditional medicine represents an urgent need. During the last decade, DNA barcoding offers an accurate and efficient strategy that can identify existing species and discover unknown species via analysis of sequence variation in a standardized region of DNA. Recent studies have shown that DNA barcoding as well as minibarcoding and metabarcoding is capable of identifying animal species and discriminating the authenticities from the adulterants in various types of traditional medicines, including raw materials, processed products, and complex preparations. These techniques can also be used to detect the unlabelled and threatened animal species in traditional medicine. Here, we review the recent progress of DNA barcoding for the identification and authentication of animal species used in traditional medicine, which provides a reference for quality control and trade supervision of animal-based traditional medicine.

1. Introduction

Traditional medicine (TM) has been widely used for the prevention and treatment of various common ailments and complicated illnesses in human history, and the use of animals as medicine, which is known as zootherapy, has long been an essential part of traditional therapeutic practices, such as Traditional Chinese Medicine (TCM), Kampo medicine, Ayurvedic medicine, and American folk medicine [1–6]. Besides the traditional usages, modern clinical studies have demonstrated that animal-based TMs possess a number of pharmacological effects, including anti-inflammatory, antitumor, anti-infective, anticonvulsant, analgesic, and immunomodulatory activities [6–9]. Therefore, zootherapy continues to serve as an important complementary and alternative therapy in modern societies. For example, over 1,500 animal species have medicinal benefits according to the historical records in China, and approximately 77 kinds of medicinal animals and 50 kinds of medicinal materials derived from animal sources have been included in the Chinese Pharmacopoeia 2015 Edition [1, 10]. In Brazil and Latin America, at least 354 and 584 animal species have been reported to be used in TM, respectively [4, 5]. It is estimated that animal-derived TM has been increasingly used in many countries and its annual global trade accounts for billions of dollars [2]. Moreover, recent studies have revealed that animal-based TM contains a variety of bioactive compounds, which provides a valuable chemical library for drug discovery [11], indicating the potential of medicinal animals in developing modern pharmaceuticals.

During recent centuries, a wide range of wild animal species is known to be threatened with extinction; for
example, according to the latest statistics of the International Union for Conservation of Nature (IUCN) Red List of Threatened Species, the number of endangered and critically endangered animal species has reached 7597 and 5101, respectively [12]. Such rapid loss of biodiversity is closely associated with animal habitat loss and human overexploitation. For instance, despite some parts such as horns and secretions, almost all types of tissues and organs used in TM require animal sacrifice, which inflicts substantial stress to medicinal animal resources [2, 13, 14]. To solve the species crisis, the trade of threatened animals has been regulated under international legislation including the Convention on International Trade in Endangered Species (CITES), and the therapeutic application of such species has been forbidden in many countries. However, some countries such as those in East and Southeast Asia still utilize threatened animals for medicinal purposes [2, 15], and the illegal trade of medicinal animals further impairs biodiversity [16, 17]. Moreover, a variety of adulterants and counterfeits has emerged in the market for greater interests, which brings challenges to the safety of animal-based TM. Therefore, identification and authentication of animal species is of vital importance for TM trade supervision and quality control.

It is known that animal-based TM are mainly derived from animal tissues, organs, or metabolites, and these parts are usually processed into diverse preparations such as slice, powder, and tablet, resulting in difficulties for morphology-based species identification. In addition, several commercial technologies used for species identification, including chromatography and immunoassay, are relatively costly and expertise-dependent, which is not suitable for bulk detection of TM preparations and forensic specimens [18, 19]. Therefore, developing an accurate and efficient identification method represents an urgent need for animal-based TM. In recent decades, advances in molecular techniques have promoted the application of simple and precise DNA analysis in taxonomic fields. Among the prevailing genome-based approaches, DNA barcoding provides a robust strategy to identify existing species and discover unknown species via comparative analysis of sequence variation [19–21]. Extensive studies have shown that DNA barcoding is capable of identifying a wide range of animal species, including mammals, birds, reptiles, amphibians, fishes, and crustaceans [21–24]. Moreover, many international organizations such as the Consortium for the Barcode of Life (CBOL) and the Barcode of Life Data System (BOLD) have been established to promote DNA barcoding as a global standard for species identification. In addition to taxonomy, DNA barcoding has recently been applied in various fields, including medicine and food science, forensics, and conservation biology [19, 20, 25–27]. In the field of TM, DNA barcoding has been widely used to authenticate herbal sources [19, 20]. However, studies on identifying medicinal animal species including the threatened species and discriminating the authentic TM from the adulterants are relatively less addressed. Therefore, we tend to review the general process and current progress of DNA barcoding in analyzing animal-based TM and then discuss its limitations and potential strategies.

2. Animal Identification by DNA Barcoding

2.1. DNA Barcoding and Minibarcoding. The accurate species identification by DNA barcoding relies on a suitable DNA barcode, which refers to a standardized sequence (usually less than 1,000 bp) of the genome [21]. The barcode should have universality so that it can be easily amplified from diverse species, and it should contain few insertions or deletions to facilitate sequence alignment. Also, its mutation rate must be sufficient to generate a barcoding gap, which means the maximum intraspecific variation is less than the minimum interspecific distance [21]. For animal identification, the most broadly used barcode marker is mitochondrial cytochrome c oxidase subunit I (COI), which is highly conserved across species employing oxidative phosphorylation for metabolism [22]. Numerous studies have shown that COI-based DNA barcoding can delimit diverse animal species, indicating the high rates of sequence change at species level and constraints on infraspecific divergence in COI sequence [21–24]. For analyzing fresh and well-preserved animal tissues, a full-length barcode such as a 658-bp region of COI gene is recommended as its PCR amplification and sequencing can be feasible. However, animal tissues are usually processed before used as TM materials, and some processing approaches, such as sun-drying, stir-frying, and boiling, can cause DNA degradation, leading to difficulties for PCR amplification of full-length barcodes [19]. Since the amplification feasibility improves with reduced sequence length, a minibarcoding method, which utilizes a shorter region within the standard barcode, has received increasing attention [28]. Recent studies have shown that an array of minibarcodes can be effectively amplified and sequenced from various processed products including TM and foods and provide sufficient sequence information necessary for species identification [26, 29, 30].

2.2. DNA Metabarcoding. It is known that many TM preparations are composed of multiple materials derived from diverse animal and/or plant species. In such mixtures, multiple barcode sequences will be coamplified and the conventional sequencing will generate multiple or overlaying sequencing peaks, resulting in ambiguity or false sequence information [31]. Therefore, DNA metabarcoding has been proposed to identify multiple species within complex samples using next-generation sequencing (NGS) technology [32, 33]. NGS can rapidly yield millions of DNA reads and obtain all representative sequences presented in mixtures, which facilitates a high-throughput multitaxa identification. This technology can also be used for sequencing minibarcodes (approximately 50–400 bp), which makes it suitable for analyzing processed samples with degraded DNA [19]. For example, Arulandhu et al. [34] have shown that a multilocus DNA metabarcoding, including a set of full-length barcodes and minibarcodes combined with Illumina MiSeq amplicon sequencing, can reproducibly identify animal and plant species present in mixtures with both low and high complexity at 1% dry weight content, demonstrating its accuracy and sensitivity in analyzing complex samples. Due to these advantages, DNA metabarcoding has been widely applied in biodiversity research, ecological management, and
community analysis [33, 35]. Recently, it has also been used to identify animal species in medicinal preparations, foods, and forensic specimens [34, 36–38]. For instance, Coghlan et al. [36] used mitochondrial 16S ribosomal RNA (16S rRNA) barcode and NGS technology to detect species in 15 complex TCMs presented in the form of powders, capsules, and tablets and revealed that some of them contained CITES-listed animal species and unlabeled species, demonstrating the capability of DNA metabarcoding in analyzing TM formulae.

3. General Process of Animal Identification by DNA Barcoding

Although diverse animal tissues and animal-based TMs are used in different studies, the main procedures of DNA barcoding are similar. After extraction of DNA from the tested samples, appropriate DNA barcodes must be selected and then amplified via the polymerase chain reaction (PCR). The amplified regions can be sequenced by conventional methods or NGS according to the sample complexity and then matched to existing sequences from reference database or voucher specimens. Further comparative analysis can be performed to detect the intraspecific and interspecific sequence divergences (Figure 1).

3.1. DNA Extraction. Extraction of DNA with high yield and quality is a crucial premise for DNA barcoding of animal species. For some types of animal tissues that contain a small amount of DNA, appropriate sampling and sufficient homogenization are important before DNA extraction. The extraction protocol usually involves several critical steps, including tissue lysis, removal of impurities from DNA, and DNA precipitation [39]. There are many classical methods for extracting out DNA from animal tissues, such as Sodium Dodecyl Sulfate (SDS) extraction, guanidinium thiocyanate-phenol-chloroform extraction, silica matrix-based purification, and magnetic bead-based purification [40]. In many laboratories, these conventional methods have been modified to improve DNA extraction efficiency; for example, Ivanova et al. [41] developed an inexpensive and automation-friendly animal DNA extraction protocol, which employed SDS and proteinase K for tissue lysis followed by silica-based purification using glass fiber filtration plates. In addition, most of these protocols have been developed into commercial kits, which provide standard DNA extraction means for different studies. As different types of animal tissues have distinct characteristics, it is necessary to select appropriate extraction methods or commercial kits (Table 1).
| Commercial kit                                      | Purification method                  | Supplier           | Starting material                     | Advantage                                                                                                                                 |
|----------------------------------------------------|--------------------------------------|--------------------|---------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------|
| Qiagen DNeasy Blood and Tissue Kit                 | Silica-based technology              | QIAGEN             | Animal tissues and blood               | Optimized protocols for a range of tissues, 96-well high-throughput formats                                                            |
| QIAamp DNA Stool Mini Kit                          | Silica-based technology              | QIAGEN             | Fresh or frozen stool                  | No organic extraction or alcohol precipitation, complete removal of contaminants and inhibitors                                         |
| TIANamp Marine Animals DNA Extraction Kit          | Silica-based technology              | TIANGEN Biotech    | Tissues of fish, shrimp, shellfish, crab, etc. | Specially developed for marine animals, no organic extraction                                                                            |
| NucleoSpin® DNA RapidLys Kit                       | Silica-based technology              | Macherey-Nagel     | Fresh, frozen, dried or ethanol preserved animal organs, eukaryotic cells, tail and ear clippings | Powerful lysis to efficiently release genomic DNA, superior genomic DNA yields                                                           |
| NucleoSpin® DNA Insect                             | Silica-based technology and NucleoSpin® Bead Tubes Type D | Macherey-Nagel     | Fresh, frozen, dried or ethanol preserved insect or crustacean | NucleoSpin® Bead Tubes for efficient lysis of an exoskeleton                                                                                   |
| NucleoSpin® DNA Lipid Tissue                       | Silica-based technology and NucleoSpin® Bead Tubes Type D | Macherey-Nagel     | Fresh or frozen, lipid-rich tissue (e.g., brain, adipose tissue, fatty fish tissue) | Special buffer for efficient removal of lipids, NucleoSpin® Bead Tubes for efficient lysis                                              |
| Non-organic DNA Extraction Kit                     | Salting out precipitation            | Merck millipore    | Whole blood, body fluid, bone marrow, mononuclear cells, solid tissues | A simple and non-toxic way to isolate high molecular weight genomic DNA                                                                  |
| OmniPrep™ kit                                      | Unique precipitation reagents        | G-Biosciences      | Tissues from different species including animal, plant, bacteria, yeast and fungus | High yield of ~800 kb genomic DNA, no organic extraction                                                                                 |
| MagAttract Blood DNA/RNA Kit                       | Magnetic bead-based technology       | MoBio              | Fresh whole blood, frozen whole blood,uffy coat, plasma | Works with all blood types, novel ClearMag magnetic particle technology                                                                 |
| ChargeSwitch® gDNA Mini Tissue Kit                 | Magnetic bead-based technology       | Invitrogen         | Animal tissues such as tail, spleen, bone and hair | High-purity DNA extraction, improved performance with accompanying magnetic rack                                                        |
| MagListo™ 5 M Forensic Sample DNA Extraction Kit    | Magnetic bead-based technology       | Bioneer            | Whole blood, saliva, urine, hair, finger nail, bone | Suitable for forensic samples, a rapid and cost-effective DNA extraction                                                                  |
3.2. Selection of Barcode Region. Extensive studies have demonstrated the capability of COI region in animal taxonomy; however, this barcode still has several limitations [38, 42]. For instance, COI barcoding region has been found to offer insufficient and unreliable discrimination for some species in the class Gastropod and Anthozoa [43, 44]. In fact, several other genes, such as mitochondrial cytochrome b (Cytb), 16S rRNA, 12S ribosomal RNA (12S rRNA), and nuclear ribosomal internal transcribed spacer (ITS), have also been used for barcoding of animal species [42, 45, 46]. For example, the ITS2 region can achieve an identification rate of 91.7% at the species level among 12,221 kinds of animals recorded in GenBank and differentiate some animal species such as the Argasidae that can be not identified by COI barcode [45]. On the other hand, minibarcodes usually exhibit higher success rate of amplification than full-length barcodes in highly processed samples. However, since the success of taxonomic differentiation is positively correlated with the barcode length, the minibarcode length is usually kept above 100 bp. For example, an approximately 250-bp region of 16S rRNA can be successfully amplified from various medicinal preparations and food products and further provides correct identification of animal species [34, 36, 47]. In addition, as a single DNA barcode may generate insufficient or false identification of hybrid species as well as animal species with high diversity [48], multilocus barcodes can be used to improve the identification accuracy and sensitivity. The DNA barcoding guideline for molecular identification of TCM, which is included in the Appendix of Chinese Pharmacopoeia, has proposed a comprehensive animal identification using both COI and ITS2 barcodes [10].

3.3. PCR Amplification of Barcode Region. The amplification efficiency of the barcode region is closely associated with the primer pairs; for example, appropriate barcode primers should be versatile across a wide range of animal species and have high affinity to DNA templates and a balanced melting temperature [49]. Ivanova et al. [50] have developed universal primer sets for amplifying COI barcode. When the universal primers are not applicable for certain taxa or specimens, it is necessary to redesign primers, such as those for minibarcodes [19, 47]. Many molecular biology software and related websites, including Primer Premier, Oligo, and Whitehead, can be used to design and evaluate primers. In addition to the primers, PCR reaction system also contains other necessities including heat-stable DNA polymerases, dNTP mixtures, and DNA templates. The reaction parameters, such as the temperature and time of melting and annealing processes, play an important role in the selective amplification of target templates, and these parameters should be optimized according to the specific circumstances [51].

3.4. Sequencing of Amplified Region. As a widely used DNA sequencing method, Sanger dideoxy sequencing is capable of generating sequencing reads up to 1,000 bp [52]. However, Sanger sequencing is low throughput, which makes it suitable for DNA barcoding of single species at a small scale [31, 53]. In contrast, NGS technology can parallelly sequencing multiple DNA fragments in a single reaction [38]. The 454 pyrosequencing is the first commercially available NGS and has been used to analyze various types of mixtures, including environmental specimens, food products, and medicinal preparations [36, 38, 54, 55]. Recently, a number of benchtop sequencers, such as Roche 454 GS Junior System, Ion Proton System, and Illumina MiSeq and MiniSeq, have been developed for routine tests in the laboratory [34, 36, 38, 56]. As NGS may produce sequencing errors, quality filtering and trimming of raw reads must be performed to remove erroneous data before barcoding analysis.

3.5. Reference Database. The accurate identification of animal species depends on the availability of reference sequence data, which are currently deposited in many public libraries, including GenBank, BOLD, Medicinal Materials DNA Barcode Database (MMDBD), International Nucleotide Sequence Database Collaboration (INSDC), and Barcode Index System (BIN). These databases contain a variety of sequences assigned to corresponding taxa, which is useful for comparative analysis of sequence variations. For example, GenBank, a commonly used database in barcoding studies, has included more than 1 Terabase sequence data with relatively broad taxon coverage [115]. Another database BOLD has already collected more than 2 million COI sequences from about 170,000 species, and INSDC has recorded extensive Cytb and ITS sequence information [38]. In addition, MMDBD focuses on the barcode information of medicinal plants and animals (over 1,700 species) listed in the Chinese Pharmacopoeia, American Herbal Pharmacopoeia, and other related references. Interestingly, MMDBD also includes sequence information of common adulterants and substitutes [116].

3.6. Sequence Analysis. Several comparative methods, including similarity-, distance-, and tree-based approaches, have been widely used to analyze sequence variations [38, 117]. The Basic Local Alignment Search Tool (BLAST) is a similarity-based algorithm, which matches the query sequence to those in reference databases and then provides a similarity score according to the portion of the query aligned to the reference [118]. For distance-based analysis, Kimura 2-parameter (K2P) model can be used to calculate the intraspecific and interspecific genetic distances among sequences, and then the barcoding gap can be used for species delimitation [117, 119]. In addition, tree-based methods are often used to establish the phylogenetic relationships, which assign query sequences to species on the basis of their membership of clusters in a barcode tree. The closest relative animal species will appear in a cluster, while distinct species should form discreet clusters. Several hierarchical clustering algorithms, including neighbor joining (NJ), maximum likelihood (ML), maximum parsimony (MP), and Bayesian inference (BI), have been used to establish phylogenetic tree, and a combination of these algorithms can provide more reliable identification compared with a single algorithm [22, 117, 120, 121]. A number of commercial tools, such as MEGA, PHYLIP, and PAUP, can be used for tree construction and visualization.
4. Authentication of Animal-Based Traditional Medicine by DNA Barcoding

It is estimated that the animals used in TM are mainly from several phyla, including Chordata, Arthropoda, Echinodermata, Annelida, Mollusca, and Coelenterata. The authentication of genetic composition is important for quality control of animal-based TM and trade regulation of threatened medicinal animals. Recently, DNA barcoding as well as minibarcoding and metabarcoding has been successfully applied to identify single (Table 2) and multiple species (Table 3) in various types of animal-based TMs and discriminate the authenticity of the adulterants [34, 36, 57–114].

4.1. Identification of Single Species

4.1.1. Phylum Chordata-Derived Traditional Medicine. The phylum Chordata used in TM mainly includes mammals, reptiles, fishes, and amphibians. Among these vertebrates, many kinds of wild mammals as well as their organs and tissues, such as horns, scales, muscles, and gallbladders, have long been an important source of TM materials in many countries, especially those in Asia and Africa [2, 122]. For example, the horns from a migratory ungulate Saiga tatarica have been used in TM for thousands of years, but the wild population of S. tatarica has rapidly declined in recent decades due to persistent hunting [57, 58]. CITES has listed this antelope in Appendix II since 1995, and the market trade of Saiga horns has been rigorously monitored. However, the horns from other species such as Capra hircus and Procapra gutturosa have been sold as Saiga horns in the market [57]. It is difficult to discriminate these horns as they share similar appearance, especially when they are processed in slices or powders. To distinguish the authenticity from the adulterants, Chen et al. [57] recovered a 644 bp region of COI gene from well-preserved horns with specific primers and a 349 bp fragment from degraded horns with nested primers. Further analysis using K2P model and NJ tree method revealed that the mean intraspecific genetic distances of both full-length barcode and minibarcode are far less than the mean interspecific distances, and S. tatarica and its adulterant species can form independent clades in phylogenetic tree. Another famous horn-based TCM is Pilos antler, which is used for many illnesses including impotence, arthritis, and anemia [123]. It is officially derived from velvet antlers of Cervus nippon and Cervus elaphus according to the Chinese Pharmacopoeia [10], while the antlers of other deer such as Rangifer tarandus and Dama dama are often sold as the genuine products [62]. Recently, several studies have shown that using a set of DNA barcodes, including COI, Cytb, and 16S rRNA, can identify the biological origins of various animal horn samples and discriminate Pilos antler from its adulterants with high sensitivity [58, 62, 64]. Together, these results demonstrate that DNA barcoding and minibarcoding are capable of authenticating horn-based TM. Moreover, barcoding techniques have been used to analyze TM derived from other mammalian tissues, such as pangolin scales, deer musk, bear bile, and donkey-hide gelatin [36, 65–70]. For instance, pangolin scales, a rare TM used for many conditions including asthma and rheumatism, are derived from Manis pentadactyla according to the Chinese Pharmacopoeia [10]. However, the scales of other pangolin species often cause market confusion due to the scarcity of effective detection methods, and the illegal pangolin trade has escalated globally in recent years despite the legislation that all pangolin species have been listed in CITES Appendix II since 1994. To identify several batches of confiscated pangolin remains in Philippines and Hong Kong, several studies used COI and Cytb barcodes and found that barcoding approach can accurately assign unknown scales to specific species and distinguish different pangolin species [66, 67], indicating that DNA barcoding is a useful strategy for customs to suppress illegal trade of threatened mammals.

It is reported that many reptiles, including snakes, geckos, and turtles, play an important role in traditional folk medicine worldwide, and these reptile-based TMs have various therapeutic benefits, such as anti-inflammatory, sedative, and analgesic effects [124]. To identify the medicinal reptiles that exhibit similar morphology interspecifically, DNA barcoding provides a simple but reliable strategy (Table 2). For example, Zaocys dhumnades and Bungarus multicinctus are two important snakes used in TCM, and their dried bodies without the viscera have been widely applied to treat several disorders including rheumatoid arthritis, stroke, and convulsion [73–77]. However, many other snake species are marked as Z. dhumnades and B. multicinctus in the market, and the accurate identification of these snake-based TMs highly relies on professional experience. Interestingly, several recent studies have used a panel of full-length barcodes and minibarcodes including COI, 12S rRNA, 16S rRNA, and Cytb to analyze various snake specimens and related TMs collected from the wild and markets in China. The results showed that each sample can be identified as specific snake species, and Z. dhumnades and B. multicinctus as well as their adulterants can be clearly distinguished at the species level [73–77], indicating the efficacy of DNA barcoding and minibarcoding in authentication of snake-based TM. Another example is the dried body of Gekko gecko, which is traditionally used for relieving coughing and asthma [72]. Using 150-bp and 648-bp fragments of COI sequence, G. gecko specimens and their adulterants such as G. japonicas and Calotes versicolor have been found to exhibit significant barcoding gaps [72], further demonstrating the capacity of barcoding techniques in analyzing reptile-based TM.

As an indispensable group of vertebrates in aquatic animals, fish provides an enormous resource for humans as foods and medicines. Modern research has revealed that many fish species, especially marine fish, contain various substances with nutritional and pharmacological benefits [125]. For example, shark fins are not only the main constituent of the delicacy shark fin soup in Asian cuisines but also the precious TCM materials used for arthritis treatment and immune enhancement [97, 126]. Although twelve shark species have been listed in Appendix II of CITES, overfishing and illegal trade continue to induce a rapid decline of shark populations. In response to this issue, DNA barcoding has recently been adopted to authenticate shark species in the market [94–98]. For processed shark products, degraded
| Medicinal species | Medicinal part | Therapeutic benefit | Adulterant species | Tested sample | Tested barcode | Reference |
|-------------------|----------------|---------------------|--------------------|---------------|----------------|-----------|
| *Saiga tatarica* | Horns | Treat fever, headaches, eye ailments, convulsion, epilepsy and agitation | *Capra hircus*, *Ovis aries*, *Procapra gutturosa*, and other ungulate species | Horns, muscles, skin | 349 bp, 487 bp and 644 bp of COI | Chen et al. [57], Yan et al. [58], Cao et al. [59] |
| *Bubalus bubalis* | Horns | Treat fever, delirium, hemoptysis and convulsion | *Bos grumii*, *Bos taurus domesticus* | Horns | 487 bp of COI | Yan et al. [58] |
| *Ceratotherium simum**, *Diceros bicornis***, *Rhinoceros unicornis**, and other rhino species* | Horns | Treat fever, influenza, convulsion, delirium, hemoptysis and abscess | *Bubalus bubalis*, *Bos taurus*, *Equus ferus*** | Horns, bones, hair, blood, feces, skin | 230 bp of Cytb | Ewart et al. [60] |
| *Cervus elaphus*, *Cervus nippon* | Velvet antlers, tendons | Treat impotence, arthritis, anemia, knee weakness, skin ailments and urinary disorders, improve muscle tone and nerve function | *Elaphurus davidianus*, *Dama dama**, Range* *tarandus*, *Bos spp.*, *Bubalus bubalis* | Antlers, tendons | COI, Cytb, 12S rRNA, 16S rRNA | Yan et al. [58], Chung et al. [61], Sin et al. [62], Jiang et al. [63], Cai et al. [64] |
| *Moschus berezovskii* | Musk | Treat stroke, coma, convulsion and sores | Skin, blood, muscles | 627 bp of COI, 723 bp of D-loop | Yang et al. [65] |
| *Manis pentadactyla* | Scales | Treat asthma, rheumatism, arthritis and stomach ulcers, enhance microcirculation | *Manis culiimensis***, *Manis javanica***, *Manis tricuspi***, and other pangolin species* | Scales, muscles, blood | ~650 bp of COI, 400 bp of Cytb, 576 bp of D-loop | Luczon et al. [66], Mwale et al. [67] |
| *Equus asinus* | Gelatin | Promote hematopoiesis and arrest bleeding | *Equus caballus*, *Bos taurus*, *Sus scrofa* | Glue, hair, muscles | <100 bp of Cytb | Kumeta et al. [68] |
| *Ursus thibetanus* | Bile, gallbladders | Treat fever, epilepsy and haemorrhoids, alleviate inflammation and pain | *Ursus americanus* | Bile, coats | COI, Cytb, 16S rRNA | Coghlan et al. [36], Janjua et al. [69], Peppin et al. [70] |
| Medicinal species          | Medicinal part           | Therapeutic benefit                                                                 | Adulterant species                                      | Tested sample | Tested barcode | Reference               |
|---------------------------|--------------------------|--------------------------------------------------------------------------------------|----------------------------------------------------------|---------------|----------------|-------------------------|
| *Panthera tigris*         | Bones                    | Relieve pain and convulsion                                                          | *Canis lupus familiaris*                                 | Tanned leathers | Cytb            | Jun et al. [71]         |
| *Lutra sumatrana*         | Skin                     | Treat fever                                                                          |                                                          | Tanned skin   | COI             | Janjua et al. [69]      |
| Chordata: Reptilia        |                          |                                                                                      |                                                          |               |                |                         |
| *Gekko gecko*             | Dried body without viscera | Treat cough, cold, impotence, asthma and tuberculosis                                | *Calotes versicolor*, *Gekko japonica*, etc.             | Muscles, livers | 648 bp and 150 bp of COI | Gu et al. [72]          |
| *Zaocys dhumnades*,       | Dried body without viscera | Treat sores, abscesses, eye infection, rheumatoid arthritis and stroke, relieve pain, spasms and convulsion | *Agkistrodon acutus*, *Coeleognathus radiates*, *Cyclophiops major*, *Dinodon rufozonatum*, *Elaphocornis carinata*, and other snake species | Muscles, other tissues, concentrated granules | 48–658 bp of COI, 142–460 bp of 16S rRNA, 183–308 bp of Cytb, 83–350 bp of 12S rRNA | Jiang et al. [73], Cao et al. [74], Wang et al. [75], Li et al. [76], Chao et al. [77] |
| *Bungarus multicinctus*   |                          |                                                                                      |                                                          |               |                |                         |
| *Chelonia mydas*          | Shell                    | Treat vertigo, agitation and insomnia                                                | Blood, tissues                                           | 815 bp of COI  |                | Naro-Maciei et al. [78] |
| *Pelodiscus sinensis*     | Shell                    | Treat vertigo, agitation and insomnia                                                | Blood, tissues                                           | 621 bp and 650 bp of COI |                | Kundu et al. [79], Reid et al. [80] |
| *Caiman crocodilus*       | Muscles                  | Treat rheumatism, asthma, epilepsy and stroke                                        | Blood, tissues                                           | 645 bp and 750 bp of COI, 1290 bp of 16S rRNA |                | Meganathan et al. [81], Jogayya et al. [82], Eaton et al. [83] |
| *Gavialis gangeticus*     | Muscles                  |                                                                                      |                                                          |                |                |                         |
| Chordata: Actinopterygii  |                          |                                                                                      |                                                          |                |                |                         |
| *Hippocampus spp.*        | Dried body without viscera | Treat impotence, asthma, insomnia, infections and sores                              | Fins                                                     | 653 bp of COI, 1140 bp of Cytb |                | Chang et al. [84], Hou et al. [85] |
| *Syngnathoides biaculeatus*, *Solegnathus hardwickii*, *Syngnathus acus* | Dried body | Treat impotence, incontinence, asthma and arteriosclerosis                            | *Doryichthys boaja*, *Hipposicyclanospilus*, *Pegasus voitans*, and other pipefish species | Muscles, fins | 649 bp of COI, ~385 bp of 12S rRNA | Zhang et al. [86], Gao et al. [87] |
| Medicinal species         | Medicinal part | Therapeutic benefit                                                                 | Adulterant species                      | Tested sample   | Tested barcode                                      | Reference                      |
|--------------------------|----------------|--------------------------------------------------------------------------------------|-----------------------------------------|-----------------|-----------------------------------------------------|-------------------------------|
| *Epinephelus* spp.       | Muscles        | Alleviate hypertension and hyperglycemia                                             | Fins, livers, muscles                   | ∼700 bp of COI  |                                                     | Torres et al. [88]            |
| *Oncorhynchus* spp., *Salmo* spp. | Muscles        | Alleviate hypertension and hyperglycemia                                             | Tissues                                 | 109–650 bp of COI |                                                     | Rasmussen et al. [89]         |
| *Scomber* spp.           | Muscles        | Relieve fatigue and nervousism                                                        | Muscles                                 | 226 bp of Cytb   |                                                     | Botti and Giuffra [90]        |
| *Decapterus Maruads*     | Muscles        | Treat dysentery and hemoptysis                                                        | Tissues                                 | 652 bp of COI   |                                                     | Mat Jaafar et al. [91]        |
| *Chordata: Chondrichthyes* | Gill rakers    | Relieve arthritis, asthma, children measles and skin sores and boils                  | Gill rakers                             | 652 bp and 761 bp of COI, ∼500 bp of 16S rRNA, 1033 bp of NADH2 |                                                     | Asis et al. [92] Zeng et al. [93] Steinke et al. [94] |
| *Allopias vulpinus*, *Allopias pelagicus*, *Carcharhinus falciformis*, *Rhincodon typus*, *Sphyrna zygaena*, and other shark species* | Fins           | Treat arthritis, improve immune system function                                       | Fins, fin soup, muscles, liver oil, skin-care products | ∼110–650 bp of COI, ∼500 bp of 16S rRNA |                                                     | Steinke et al. [94] Liu et al. [95] Chuang et al. [96] Fields et al. [97] Cardeñoso et al. [98] |
| *Chordata: Amphibia*     |                |                                                                                      |                                         |                 |                                                     |                               |
| *Rana temporaria*        | Dried body, oil| Treat throat inflammation, cough, asthma and arthritis                               | Tissues                                 | 16S rRNA        |                                                     | Maya-Soriano et al. [99]      |
| *Bufo gargarizans*       | Toad cake, skin| Treat heart diseases, skin ailments and some cancers                                  | Toe tips, muscles, livers               | 565–602 bp of COI|                                                     | Che et al. [100]              |
| *Andrias davidianus***   | Dried body, skin| Treat anemia, dysentery, chills and burns                                             | Toe tips, muscles, livers               | 565–602 bp of COI|                                                     | Che et al. [100]              |
| *Batrachuperus pinconii*  | Dried body, skin| Treat rheumatism and stomachache                                                      | Toe tips, muscles, livers               | 565–602 bp of COI|                                                     | Che et al. [100]              |
| *Arthropoda: Insecta*    |                |                                                                                      |                                         |                 |                                                     |                               |
| *Chrysomya megacephala*  | Dried larvae   | Treat malnutrition and skin and soft tissue wounds                                   | *Lucilia illustris*, *Musca sorbens*, and other fly species | Hind legs       | 658 bp of COI                                       | Qiu et al. [101]              |
| Medicinal species          | Medicinal part                          | Therapeutic benefit                                                                 | Adulterant species                                                                 | Tested sample       | Tested barcode             | Reference                  |
|---------------------------|-----------------------------------------|-------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|---------------------|-----------------------------|----------------------------|
| Cordyceps sinensis        | Hepialidae caterpillars and fungal stromata | Treat asthma, bronchitis, erectile dysfunction, diabetes, cough, cold and jaundice | Cordyceps gunnii, Cordyceps gracilis, Cordyceps hawkesii, and other Cordyceps species | Stromata            | 146–493 bp of ITS          | Xiang et al. [102]          |
|                           |                                         |                                                                                     |                                                                                     |                     |                             | Lam et al. [103]            |
|                           |                                         |                                                                                     |                                                                                     |                     |                             | Liu et al. [104]            |
| Arthropoda: Arachnida     |                                         |                                                                                     |                                                                                     |                     |                             |                            |
| Mesobuthus martensi       | Dried body, venom                       | Treat rheumatoid arthritis, stroke, epilepsy, multiple sclerosis, chronic pains and some cancers | Mesobuthus gibbosus, Mesobuthus eunicus                                           | Tissues, concentrated granules | 48–94 bp of COI, 83–350 bp of 12S rRNA, 142–460 bp of 16S rRNA | Jiang et al. [73]           |
|                           |                                         |                                                                                     |                                                                                     |                     |                             | Ortiz et al. [105]          |
| Arthropoda: Malacostraca  |                                         |                                                                                     |                                                                                     |                     |                             |                            |
| Charybdis lucifera, Portunus pelagicus, Scylla serrata, and other crab species | Shell                              | Treat cancer, inflammatory bowel disease and chilblain                             | Muscles                                                            | Muscles              | 650 bp of COI               | Vartak et al. [106]         |
|                           |                                         |                                                                                     |                                                                                     |                     |                             | Mirzapur et al. [107]       |
| Echinodermata: Holothuroidea |                                         |                                                                                     |                                                                                     |                     |                             |                            |
| Holothurian species       | Whole body                              | Treat joint pain, arthritis, tendonitis and sprain                                 | Tube feet, arms, gonads                                                           | Tube feet, arms, gonads | 528 bp and 657 bp of COI   | Uthicke et al. [108]        |
|                           |                                         |                                                                                     |                                                                                     |                     |                             | Ward et al. [109]           |
| Annelida: Clitellata      |                                         |                                                                                     |                                                                                     |                     |                             |                            |
| Eisenia fetida            | Dried body                              | Treat fever and convulsion                                                          | Tissues                                                            | Tissues              | 581 bp of COI               | Rombke et al. [110]         |
| Mollusca: Bivalvia         |                                         |                                                                                     |                                                                                     |                     |                             |                            |
| Crassostrea rivularis, Crassostrea gigas | Shell                          | Treat impotence, insomnia, epilepsy, bone loss and ulcer                        | Hyotissa hyotis, Ostrea adults, and other oyster species                      | Muscles              | COI, 16S rDNA               | Hsiao et al. [111]          |
|                           |                                         |                                                                                     |                                                                                     |                     |                             | Liu et al. [112]            |
| Cnidaria: Anthozoa        |                                         |                                                                                     |                                                                                     |                     |                             |                            |
| Corallium rubrum          | Skeleton                                | Treat arthritis and ulcer                                                           | Other coral species                                                                 | Fragments           | 1597 bp of COI              | Uda et al. [113]            |

* Included in the Appendices of the Convention on International Trade in Endangered Species (CITES); † Recorded as endangered, critically endangered or extinct in the wild status in the International Union for Conservation of Nature (IUCN) Red List of Threatened Species.
| Tested sample | Therapeutic benefit | Barcode | Sequencing method | Labeled animal species | Identified animal species | Reference |
|---------------|---------------------|---------|-------------------|------------------------|--------------------------|-----------|
| Ma pak leung sea-dog hard capsules | Enhance sexual and physical function | 12 barcodes | Illumina MiSeq amplicon | Cervus sp. | Bos taurus | Arulandhu et al. [34] |
| Cobra performance enhancer hard capsules | Enhance sexual and physical function | 12 barcodes | Illumina MiSeq amplicon | Hippocampus sp.*, Callorhinus sp. | Bos taurus, Homo sapiens | Arulandhu et al. [34] |
| Mongnan Tianbao Pills | Enhance sexual and physical function | 16S rRNA | Roche GS Junior | Saiga tatarica** | Saiga tatarica*, Capra sp. Ovis sp. | Coghlan et al. [36] |
| Ling Yang Ge Gen Cold Remedy | Relieve fever, cold and influenza | 16S rRNA | Roche GS Junior | Saiga tatarica** | Saiga tatarica**, Capra sp. Ovis sp. | Coghlan et al. [36] |
| Laryngitis Pills | Treat throat sores, acute tonsillitis and mumps | 16S rRNA | Roche GS Junior | Ursus sp.*, Anser sp. | Ursus thibetanus* | Coghlan et al. [36] |
| Yatong Yili Wan Powder | Relieve gingival pain | 16S rRNA | Roche GS Junior | Canis sp., Panthera uncia*, Panthera tigris** | Canis sp., Rattus sp., Deinagkistrodon sp. | Coghlan et al. [114] |
| Powder in vials | Alleviate inflammation | 16S rRNA | Roche GS Junior | Ursus thibetanus* | Ursus thibetanus* | Coghlan et al. [36] |
| Chu pak hou tsao san | Relieve cough, reduce phlegm | 16S rRNA | Roche GS Junior | Macaca sp, Bivalvia, Bos taurus | Canis sp., Rattus sp., Deinagkistrodon sp. | Coghlan et al. [114] |
| TCM 8 | Treat arthritis and pain | 16S rRNA | Roche GS Junior | Canis sp., Panthera uncia*, Panthera tigris** | Canis sp., Rattus sp., Deinagkistrodon sp. | Coghlan et al. [114] |
| TCM 11 | 16S rRNA | Roche GS Junior | | | | |
| TCM 20, TCM 26 | 16S rRNA | Roche GS Junior | | | | |
| TCM 25 | 16S rRNA | Roche GS Junior | | | | |

* Included in the Appendices of the Convention on International Trade in Endangered Species (CITES); * Recorded as endangered, critically endangered or extinct in the wild status in the International Union for Conservation of Nature (IUCN) Red List of Threatened Species.
 genomic DNA may lead to unsuccessful amplification of full-length barcodes. Therefore, several studies used shorter fragments of COI (less than 200 bp) and showed that these minibarcodes can accurately identify CITES-listed shark species from processed fins and even fin soup and skin-care products [97, 98], demonstrating the capability of DNA minibarcoding in analyzing processed animal tissues. Another example is *Hippocampus* spp., which has a unique appearance in the family Syngnathidae and is a famous TM material used to treat impotence, asthma, and insomnia [84, 85]. As a mass of seahorses have been harvested and traded annually, all seahorse species have been included in Appendix II of CITES since 2004. To investigate the usage of seahorses in Chinese TM market, several recent studies employed DNA barcoding to analyze dried seashore specimens and revealed that both COI and Cytb barcodes can efficiently authenticate seahorse species and identify endangered species [84, 85]. Together, these studies demonstrate the availability of DNA barcoding in trade supervision of threatened fish species used in TM.

Amphibians are a group of ectothermic vertebrates characterized by their ability to exploit both aquatic and terrestrial habitats. Some of them, such as toads, frogs, and salamanders, are traditionally used to treat a number of ailments in many countries, including China, Japan, South Korea, and Spain [127, 128]. For example, *Rana temporaria* and *Bufo gargarizans* are two common amphibians used in TM. The dried body and oil derived from *R. temporaria* are traditionally used to relieve cough and asthma, while the toad cake and skin of *B. gargarizans* have been used for treating heart diseases, skin ailments, and other systemic illnesses [10, 129]. However, other frog and toad species are often counterfeited as the authenticities in the market. Recently, several studies have shown that distinct frogs and toads including *R. temporaria* and *B. gargarizans* can be clearly distinguished using DNA barcoding approach based on COI and 16S rRNA sequences [99, 100], which provides a reference for authentication of amphibian-based TM.

### 4.2. Identification of Multiple Species

Recent concerns about the safety and legality of TM have prompted more rigorous surveillance. Interestingly, several studies have shown that DNA metabarcoding is capable of authenticating labeled species and detecting undeclared taxa in animal-based TM formulations (Table 3). For example, using 16S rRNA barcode combined with Roche GS Junior sequencing, half of the TM preparations legally purchased in South Australia were found to contain DNA from undeclared animal or plant taxa [114]. Another recent study has developed a multilocus metabarcoding approach that employs 12 DNA barcode markers and Illumina MiSeq amplicon sequencing and revealed that Ma pak leung sea-dog hard capsules and Cobra performance enhancer hard capsules, both of which are used to treat sexual weakness, contain DNA from nondeclared taxa such as *Bos taurus* and *Homo sapiens* instead of labeled species [34]. Together, these studies suggest that metabarcoding can provide a pharmacovigilance measure for pre- and postmarket auditing of TM. In addition, metabarcoding has recently been used to identify threatened animal species in a variety of complex samples including TM preparations (Table 3). For instance, Coghlan et al. [114] have shown that 16S rRNA-based DNA metabarcoding can detect DNA from endangered animals *Panthera uncia* and possibly *Panthera tigris* in a TM sample used to treat arthritis and pain. Moreover, similar technique was used to audit the genetic composition of some TM samples seized by Australian Customs and Border Protection Service, and the results showed that *Saiga antelope* horn powder contained DNA from the known CITES-listed species *Saiga tatarica* and other species including goat and sheep, and Chu pak hou tsao san powder contained DNA from *Ursus thibetanus*, which is recorded in both CITES Appendix I and IUCN Red List [36]. These results suggest that DNA metabarcoding is useful for custom authority to analyze forensic specimens. Interestingly, DNA metabarcoding has also been used to identify the diet composition from animal species.
secretions, such as the floral species of honey [136], further demonstrating its ability in biodiversity analysis.

5. Limitation and Prospect

Although DNA barcoding is an effective complement to conventional identification methods, it still has a few shortcomings [42, 137]; for example, some animal tissues such as horns, shells, and scales contain relatively small amount of DNA, resulting in difficulties for template amplification. Moreover, a false identification may be generated due to the contamination in DNA extraction and PCR reaction processes. Thus it is important to sample the tissues containing more cells and make sure all procedures are standardized. For example, to obtain a high yield and quality of DNA from animal horns, 75% ethanol can be used for sterilization, and middle layer between the bone core and outer sheath can be collected and then ground into powder in liquid nitrogen [57]. On the other hand, some TM preparations such as the extracts are highly processed, inducing degradation of DNA into very small fragments or even complete removal of DNA. In such cases, it is preferred to perform DNA barcoding analysis before the raw materials are processed. Another concern for complex samples with degraded DNA is that the amplification success of a barcode region may be different for distinct species due to varied gene copy number [20, 31]. It is thus necessary to employ multilocus barcoding and minibarcoding approaches and design novel primers for certain taxa.

The feasibility and accuracy of DNA barcoding are closely associated with both reference sequence data and taxonomically confirmed specimens. Although GenBank has included extensive COI sequences in a wide range of animal species, the information of some species used in TM is still lacking, and the sequence inventory of other barcodes such as 16S rRNA and Cytb also needs to be extended [38, 42]. Moreover, it is important to improve the availability of professionally authentic vouchers in public DNA databases as the misidentified species will generate incorrect sequence information [138, 139]. In addition, although MMDBD includes thousands of TM materials, the listed animal species and their adulterants are still insufficient, and the animal species used in other TM systems are also lacking. Therefore, more related databases should be established to provide sufficient bases for DNA barcoding analysis of animal-derived TM.

The quality of animal-based TM is known to be related to multiple factors, such as the specified tissues, the effective substances and even the growth stage. DNA-based analysis is feasible for genetic authenticity but unable to evaluate the pharmacological effects of TMs [19]; for example, DNA barcoding cannot distinguish different tissues from the same animal or determine the content of bioactive substances. It also fails to detect animal growth stages while some TM materials require animals at specific stages. Moreover, DNA barcoding is not feasible to identify the adulterants that do not contain DNA. To overcome these restrictions, it is necessary to combine genetic techniques with conventional approaches such as chromatography and metabolomics. Interestingly, a recent study used multidisciplinary techniques, including NGS, high performance liquid chromatography, and mass spectrometer, and showed that some TCM samples not only contained unlabelled animal species but also had undeclared pharmaceutical agents and excess heavy metals [114], indicating the potential of comprehensive analysis system in evaluating TM quality and reducing market fraud.

6. Conclusion

DNA barcoding offers a reliable and efficient strategy for the identification of authentic animal species and their adulterants in TM. It is noteworthy that the success of DNA barcoding is related to many factors, such as high quality of DNA and appropriate barcodes. For processed animal tissues with degraded DNA, minibarcodes usually exhibit higher success rate in species identification as compared with full-length barcodes. For complex mixtures such as TM formulae, metabarcoding provides a feasible approach to simultaneously detect multiple animal ingredients. With a global accumulation of open access reference sequences, DNA barcoding gradually becomes an authoritative approach in TM authentication. Despite these advantages, DNA barcoding still has several inherent limitations, such as inability to identify medicinal parts or determine compounds with pharmacological activities. Therefore, establishing a comprehensive identification system including barcoding and other techniques will provide more information for quality assessment and trade monitor of animal-based TM.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors’ Contributions

Fan Yang and Fei Ding contributed equally to this work.

Acknowledgments

This work was supported by the Project of Institute of Forensic Science in Ministry of Public Security (Grant 2016FGKKFT03), the Natural Science Foundation of Guangdong Province (Grant 2017A030313864), and the Innovation and Strong School Project of Guangdong Pharmaceutical University (Grant 2016QJNCX084).

References

[1] Z. Liu, Z. Jiang, H. Fang et al., “Perception, price and preference: Consumption and protection of wild animals used in traditional medicine,” *PLoS ONE*, vol. 11, no. 3, Article ID e0145901, 2016.

[2] E. M. Costa-Neto, “Animal-based medicines: Biological prospection and the sustainable use of zootherapeutic resources,” *Anais da Academia Brasileira de Ciencias*, vol. 77, no. 1, pp. 33–43, 2005.

[3] A. J. Alonso-Castro, “Use of medicinal fauna in Mexican traditional medicine,” *Journal of Ethnopharmacology*, vol. 152, no. 1, pp. 53–70, 2014.
[4] R. R. N. Alves, T. P. R. Oliveira, and I. L. Rosa, “Wild animals used as food medicine in Brazil,” Evidence-Based Complementary and Alternative Medicine, vol. 2013, Article ID 670352, 12 pages, 2013.

[5] R. R. N. Alves and H. N. Alves, “The faunal drugstore: animal-based remedies used in traditional medicines in Latin America,” Journal of Ethnobiology and Ethnomedicine, vol. 7, article 9, 2011.

[6] D. Q.-H. Wang and M. C. Carey, “Therapeutic uses of animal bile in traditional Chinese medicine: an ethnopharmacological, biophysical chemical and medicinal review,” World Journal of Gastroenterology, vol. 20, no. 29, pp. 9952–9975, 2014.

[7] C.-Y. Wang, X.-Y. Bai, and C.-H. Wang, “Traditional Chinese medicine: a treasured natural resource of anticancer drug research and development,” American Journal of Chinese Medicine, vol. 42, no. 3, pp. 543–559, 2014.

[8] K. Kumaravel, S. Ravichandran, T. Balasubramanian, and L. Sonneschein, “Seahorses - A source of traditional medicine,” Natural Product Research (Formerly Natural Product Letters), vol. 26, no. 24, pp. 2330–2334, 2012.

[9] F. S. Ferreira, S. V. Brito, D. L. Sales et al., “Anti-inflammatory potential of zootherapeutics derived from animals used in Brazilian traditional medicine,” Pharmaceutical Biology, vol. 52, no. II, pp. 1403–1410, 2014.

[10] C. P. Commission, Pharmacopoeia of the Peoples Republic of China, Chemical Industry Press, Beijing, China, 2015.

[11] H. Yuan, Q. Ma, L. Ye, and G. Piao, “The traditional medicine and modern medicine from natural products,” Molecules, vol. 21, no. 5, article 559, 2016.

[12] “The International Union for Conservation of Nature (IUCN) Red List of Threatened Species,” http://www.iucnredlist.org/.

[13] V. L. Williams and M. J. Whiting, “A picture of health? Animal use and the Faraday traditional medicine market, South Africa,” Journal of Ethnopharmacology, vol. 179, pp. 265–273, 2016.

[14] R. W. Byard, “Traditional medicines and species extinction: another side to forensic wildlife investigation,” Forensic Science, Medicine and Pathology, vol. 12, no. 2, pp. 125–127, 2016.

[15] S. Rastogi and K. Kaphle, “Sustainable traditional medicine: taking the inspirations from ancient veterinary science,” Evidence-Based Complementary and Alternative Medicine, vol. 2011, Article ID 151435, 6 pages, 2011.

[16] J.-Y. Luo, D. Yan, J.-Y. Song et al., “A strategy for trade monitoring and substitution of the organs of threatened animals,” Scientific Reports, vol. 3, article 3108, 2013.

[17] M. Zhang, A. Gouveia, T. Qin, R. Quan, and V. Nijman, “Illegal pangolin trade in northernmost Myanmar and its links to India and China,” Global Ecology and Conservation, vol. 10, pp. 23–31, 2017.

[18] J. Han, X. Pang, B. Liao, H. Yao, J. Song, and S. Chen, “An authenticity survey of herbal medicines from markets in China using DNA barcoding,” Scientific Reports, vol. 6, Article ID 18723, 2016.

[19] B. Mohammed Abubakar, F. Mohd Salleh, M. S. Shamsir Omar, and A. Wagiran, “Review: DNA barcoding and chromatography fingerprints for the authentication of botanicals in herbal medicinal products,” Evidence-Based Complementary and Alternative Medicine, vol. 2017, Article ID 1352948, 28 pages, 2017.

[20] P. Mishra, A. Kumar, A. Nagireddy et al., “DNA barcoding: an efficient tool to overcome authentication challenges in the herbal market,” Plant Biotechnology Journal, vol. 14, no. 1, pp. 8–12, 2015.

[21] P. D. N. Hebert, A. Cywinska, S. L. Ball, and J. R. DeWaard, “Biological identifications through DNA barcodes,” Proceedings of the Royal Society B Biological Science, vol. 270, no. 1512, pp. 313–321, 2003.

[22] P. D. N. Hebert, S. Ratnasingham, and J. R. DeWaard, “Barcoding animal life: cytochrome c oxidase subunit I divergences among closely related species,” Proceedings of the Royal Society B Biological Science, vol. 270, supplement 1, pp. S96–S99, 2003.

[23] N. V. Ivanova, E. L. Clare, and A. V. Borisenko, “DNA barcoding in mammals,” Methods in Molecular Biology, vol. 858, pp. 153–182, 2012.

[24] M. Vences, Z. T. Nagy, G. Sonet, and E. Verheyen, “DNA barcoding amphibians and reptiles,” Methods in Molecular Biology, vol. 858, pp. 79–107, 2012.

[25] S. E. Miller, A. Hausmann, W. Hallwachs, and D. H. Janzen, “Advancing taxonomy and bioinventories with DNA barcodes,” Philosophical Transactions of the Royal Society B: Biological Sciences, vol. 371, no. 1702, Article ID 20150339, 2016.

[26] Y.-T. Lo and P.-C. Shaw, “DNA-based techniques for authentication of processed food and food supplements,” Food Chemistry, vol. 240, pp. 767–774, 2018.

[27] R. Panday, D. K. Jha, N. Thapa, B. R. Pokharel, and N. K. Aryal, “Forensic wildlife parts and their product identification and individualization using DNA barcoding,” The Open Forensic Science Journal, vol. 7, no. 1, pp. 6–13, 2014.

[28] W. J. Kress, C. García-Robledo, M. Uriarte, and D. L. Erickson, “DNA barcodes for ecology, evolution, and conservation,” Trends in Ecology & Evolution, vol. 30, no. 1, pp. 25–35, 2015.

[29] M. Song, G.-Q. Dong, Y.-Q. Zhang, X. Liu, and W. Sun, “Identification of processed Chinese medicinal materials using DNA mini-barcoding,” Chinese Journal of Natural Medicines, vol. 15, no. 7, pp. 481–486, 2017.

[30] S. Shokralla, R. S. Hellberg, S. M. Handy, I. King, and M. Hajibabaei, “A DNA Mini-Barcoding System for Authentication of Processed Fish Products,” Scientific Reports, vol. 5, Article ID 15894, 2015.

[31] I. Parveen, S. Gafner, N. Techen, S. J. Murch, and I. A. Khan, “DNA barcoding for the identification of botanicals in herbal medicine and dietary supplements: Strengths and limitations,” Planta Medica, vol. 82, no. 14, pp. 1225–1235, 2016.

[32] E. Coissac, T. Riaz, and N. Puillandre, “Bioinformatic challenges for DNA metabarcoding of plants and animals,” Molecular Ecology, vol. 21, no. 8, pp. 1834–1847, 2012.

[33] P. Taberlet, E. Coissac, F. Pompanon, C. Brochmann, and E. Willerslev, “Towards next-generation biodiversity assessment using DNA metabarcoding,” Molecular Ecology, vol. 21, no. 8, pp. 2045–2050, 2012.

[34] A. J. Arulandhu, M. Staats, and R. Hagelaar, “Development and validation of a multi-locus DNA metabarcoding method to identify endangered species in complex samples,” GigaScience, vol. 6, no. 10, pp. 1–18, 2017.

[35] M. Leray and N. Knowlton, “DNA barcoding and metadiversity of standardized samples reveal patterns of marine benthic diversity,” Proceedings of the National Academy of Sciences of the United States of America, vol. 112, no. 7, pp. 2076–2081, 2015.

[36] M. L. Coghlan, J. Haile, J. Houston et al., “Deep sequencing of plant and animal DNA contained within traditional Chinese medicines reveals legality issues and health safety concerns,” PLoS Genetics, vol. 8, no. 4, Article ID e1002657, 2012.
herbal product authentication,” *Phytochemical Analysis*, vol. 29, no. 2, pp. 123–128, 2018.

[38] M. Staats, A. J. Arulandhu, B. Gravendeel et al., “Advances in DNA metabarcoding for food and wildlife forensic species identification,” *Analytical and Bioanalytical Chemistry*, vol. 408, no. 17, pp. 4615–4630, 2016.

[39] S. A. Thatcher, “DNA/RNA preparation for molecular detection,” *Clinical Chemistry*, vol. 61, no. 1, pp. 89–99, 2015.

[40] S. C. Tan and B. C. Yiap, “DNA, RNA, and protein extraction: the past and the present,” *Journal of Biomedicine and Biotechnology*, vol. 2009, Article ID 574398, 10 pages, 2009.

[41] N. V. Ivanova, J. R. Dewaard, and P. D. N. Hebert, “An inexpensive, automation-friendly protocol for recovering high-quality DNA,” *Molecular Ecology Resources (Formerly known as Molecular Ecology Notes)*, vol. 6, no. 4, pp. 998–1002, 2006.

[42] J. Waugh, “DNA barcoding in animal species: progress, potential and pitfalls,” *BioEssays*, vol. 29, no. 2, pp. 188–197, 2007.

[43] C. P. Meyer and G. Paulay, “DNA barcoding: error rates based on comprehensive sampling,” *PLoS Biology*, vol. 3, no. 12, article e422, 2005.

[44] D. Huang, R. Meier, P. A. Todd, and L. M. Chou, “Slow mitochondrial COI sequence evolution at the base of the metazoan tree and its implications for DNA barcoding,” *Journal of Molecular Evolution*, vol. 66, no. 2, pp. 167–174, 2008.

[45] H. Yao, J. Song, C. Liu et al., “Use of ITS2 region as the universal DNA barcode for plants and animals,” *PLoS ONE*, vol. 5, no. 10, Article ID e13102, 2010.

[46] V. Pradhan, Y. Kamble, V. Ladniya, and M. Mogul, “A overview of species identification by DNA barcoding,” *International Journal of Current Microbiology and Applied Sciences*, vol. 4, no. 4, pp. 127–140, 2015.

[47] C. Sarri, C. Stamatis, T. Sarafidou et al., “A new set of 16S rRNA universal primers for identification of animal species,” *Food Control*, vol. 43, pp. 35–41, 2014.

[48] D. Rubinoff, S. Cameron, and K. Will, “A genomic perspective on the shortcomings of mitochondrial DNA for "barcoding" identification,” *Journal of Heredity*, vol. 97, no. 6, pp. 581–594, 2006.

[49] S. J. Green, R. Venkatramanan, and A. Naqib, “Deconstructing the polymerase chain reaction: understanding and correcting biases associated with primer degeneracies and primer-template mismatches,” *PLoS ONE*, vol. 10, no. 5, Article ID e0128122, 2015.

[50] N. V. Ivanova, T. S. Zemlak, R. H. Hanner, and P. D. N. Hebert, “Universal primer cocktails for fish DNA barcoding,” *Molecular Ecology Resources (Formerly known as Molecular Ecology Notes)*, vol. 7, no. 4, pp. 544–548, 2007.

[51] K. B. Mullis, “The unusual origin of the polymerase chain reaction,” *Scientific American*, vol. 262, no. 4, pp. 56–65, 1990.

[52] F. Sanger, S. Nicklen, and A. R. Coulson, “DNA sequencing with chain-terminating inhibitors,” *Proceedings of the National Academy of Sciences of the United States of America*, vol. 74, no. 12, pp. 5463–5467, 1977.

[53] M. E. Polz and C. M. Cavanaugh, “Bias in template-to-product ratios in multitemplate PCR,” *Applied and Environmental Microbiology*, vol. 64, no. 10, pp. 3724–3730, 1998.

[54] M. De Barba, C. Miquel, F. Boyer et al., “DNA metabarcoding multiplexing and validation of data accuracy for diet assessment: Application to omnivorous diet,” *Molecular Ecology Resources*, vol. 14, no. 2, pp. 306–323, 2014.

[55] A. Valenti, P. Taberlet, C. Maud et al., “Next-generation monitoring of aquatic biodiversity using environmental DNA metabarcoding,” *Molecular Ecology*, vol. 25, no. 4, pp. 929–942, 2016.

[56] F. Bertolini, M. C. Ghionda, E. D’Alessandro, C. Geraci, V. Chiofalo, and L. Fontanesi, “A next generation semiconductor based sequencing approach for the identification of meat species in DNA mixtures,” *PLoS ONE*, vol. 10, no. 4, Article ID e0121701, 2015.

[57] J. Chen, Z. Jiang, C. Li et al., “Identification of ungulates used in a traditional Chinese medicine with DNA barcoding technology,” *Ecology and Evolution*, vol. 5, no. 9, pp. 1818–1825, 2015.

[58] D. Yan, J. Y. Luo, Y. M. Han et al., “Forensic DNA barcoding and bio-response studies of animal horn products used in traditional medicine,” *PLoS ONE*, vol. 8, no. 2, Article ID e55854, 2013.

[59] M. Cao, J. Wang, L. Yao, S. Xie, J. Du, and X. Zhao, “Authentication of animal signatures in traditional Chinese medicine of Lingyang Qingfei Wan using routine molecular diagnostic assays,” *Molecular Biology Reports*, vol. 41, no. 4, pp. 2485–2491, 2014.

[60] K. M. Ewart, G. J. Frankham, R. McEwing et al., “An internationally standardized species identification test for use on suspected seized rhinoceros horn in the illegal wildlife trade,” *Forensic Science International: Genetics*, vol. 32, pp. 33–39, 2018.

[61] H.-S. Chung, H. J. Lee, Y. E. Kim et al., “Diagnostic polymorphisms in the mitochondrial DNA allow discrimination among Cervus elaphus species,” *Biochip Journal*, vol. 3, no. 3, pp. 261–265, 2009.

[62] W. Sin, Y. Tam, S. Tsui, C. Ng, C. Mok, and W. Ha, “An integrated and validated DNA-based protocol developed to fight against commercial frauds – A case of fraudulent substitutions for deer products,” *DNA Barcodes*, vol. 1, pp. 27–34, 2013.

[63] L.-L. Jiang, C.-L. Liu, Y.-L. Wong, C.-F. Nip, and P.-C. Shaw, “Differentiation of deer tendons from cattle tendons by a loop-mediated isothermal amplification (LAMP) test and bone remodeling bioassays,” *Chinese Medicine*, vol. 10, no. 1, article 33, 2015.

[64] Y. Cai, L. Zhang, Y. Wang et al., “Identification of deer species (Cervidae, Cetartiodactyla) in China using mitochondrial cytochrome c oxidase subunit I (mtDNA COI),” *Mitochondrial DNA Part A: DNA Mapping, Sequencing, and Analysis*, vol. 27, no. 6, pp. 4240–4243, 2016.

[65] C. Yang, Z. Xiao, Y. Zou et al., “DNA barcoding revises a misidentification on musk deer,” *Mitochondrial DNA*, vol. 26, no. 4, pp. 605–612, 2015.

[66] A. U. Luzcon, P. S. Ong, J. P. Quiland, and I. K. C. Fontanilla, “Determining species identity from confiscated pangolin remains using DNA barcoding,” *Mitochondrial DNA Part B: Resources*, vol. 1, no. 1, pp. 763–766, 2016.

[67] M. Mwale, D. L. Dalton, R. Jansen et al., “Forensic application of DNA barcoding for identification of illegally traded African pangolin scales,” *Genome*, vol. 60, no. 3, pp. 272–284, 2017.

[68] Y. Kumeta, T. Maruyama, H. Asama, Y. Yamamoto, T. Hakamatsuka, and Y. Goda, “Species identification of Asini Corii Collas pangolinscales,” *Journal of Natural Medicines*, vol. 68, no. 1, pp. 181–185, 2014.

[69] S. Janjua, Fakhar-I-Abbas, K. William, I. U. Malik, and J. Mehr, “DNA Mini-barcoding for wildlife trade control: a case study on identification of highly processed animal materials,” *Mitochondrial DNA Part A: DNA Mapping, Sequencing, and Analysis*, vol. 28, no. 4, pp. 544–546, 2017.

[70] L. Peppin, R. McEwing, G. R. Carvalho, and R. Ogden, “A DNA-based approach for the forensic identification of Asiatic black
bear (Ursus thibetanus) in a traditional Asian medicine," Journal of Forensic Sciences, vol. 53, no. 6, pp. 1358–1362, 2008.

[71] J. Jun, S. H. Han, T.-J. Jeong, H. C. Park, B. Lee, and M. Kwak, "Wildlife forensics using mitochondrial DNA sequences: species identification based on hairs collected in the field and confiscated tanned Felidae leathers," Genes & Genomics, vol. 33, no. 6, pp. 721–726, 2011.

[72] H.-P. Gu, Y. Xia, R. Peng, B.-H. Mo, L. Li, and X.-M. Zeng, "Authentication of Chinese crude drug gecko by DNA barcoding," Natural Product Communications (NPC), vol. 6, no. 1, pp. 67–71, 2011.

[73] L.-L. Jiang, Y.-T. Lo, W.-T. Chen, and P.-C. Shaw, "DNA authentication of animal-derived concentrated Chinese medicine granules," Journal of Pharmaceutical and Biomedical Analysis, vol. 129, pp. 398–404, 2016.

[74] S. Cao, L. Guo, H. Luo et al., "Application of COI barcode sequence for the identification of snake medicine (Zoysa)," Mitochondrial DNA, vol. 27, no. 1, pp. 483–489, 2016.

[75] Y. Wang, K. Zhou, L. Xu, T. T. X. Dong, and K. W. K. Tsim, "Authentication of an animal crude drug, Zoysa, by diagnostic PCR," Biological & Pharmaceutical Bulletin, vol. 23, no. 5, pp. 585–588, 2000.

[76] X. Li, W. Zeng, J. Liao, Z. Liang, S. Huang, and Z. Chao, "DNA barcode-based PCR-RFLP and diagnostic PCR for authentication of Jinqian Baihua She (Bungarus parvus)," Evidence-Based Complementary and Alternative Medicine, vol. 2015, Article ID 402820, 7 pages, 2015.

[77] Z. Chao, J. Liao, Z. B. Liang, S. H. Huang, L. Zhang, and Z. D. Li, "Cytochrome C oxidase subunit I barcodes provide an efficient tool for Jinqian Baihua She (Bungarus parvus) authentication," Pharmacognosy Magazine, vol. 10, no. 40, pp. 449–457, 2014.

[78] E. Naro-Maciel, B. Reid, N. N. Fitzsimmons, M. Le, R. Desalle, and G. Amato, "DNA barcodes for globally threatened marine turtles: a registry approach to documenting biodiversity," Molecular Ecology Resources, vol. 10, no. 2, pp. 252–263, 2010.

[79] S. Kundu, B. A. Laskar, K. Venkataraman, D. Banerjee, and V. Kumar, "DNA barcoding of Nilssonia congeneris corroborates existence of wild N. nigricans in northeast India," Mitochondrial DNA, vol. 27, no. 4, pp. 2753–2756, 2016.

[80] B. N. Reid, M. Le, W. P. McCord et al., "Comparing and combining distance-based and character-based approaches for barcoding turtles," Molecular Ecology Resources, vol. 11, no. 6, pp. 956–967, 2011.

[81] P. P. Meganathan, B. Dubey, K. N. Jogayya, and I. Haque, "Identification of Indian crocodile species through DNA barcodes," Journal of Forensic Sciences, vol. 58, no. 4, pp. 993–998, 2013.

[82] K. N. Jogayya, P. P. Meganathan, B. Dubey, and I. Haque, "Mitochondrial 16S rRNA gene for forensic identification of crocodile species," Journal of Forensic and Legal Medicine, vol. 20, no. 4, pp. 334–338, 2013.

[83] M. J. Eaton, G. L. Meyers, S.-O. Kolokotronis, M. S. Leslie, A. P. Martin, and G. Amato, "Barcoding bushmeat: Molecular identification of Central African and South American harvested vertebrates," Conservation Genetics, vol. 11, no. 4, pp. 1389–1404, 2010.

[84] C.-H. Chang, N.-H. Jiang-Liaw, Y.-S. Lin, Y.-C. Fang, and K.-T. Shao, "Authenticating the use of dried seahorses in the traditional Chinese medicine market in Taiwan using molecular forensics," Journal of Food and Drug Analysis, vol. 21, no. 3, pp. 310–316, 2013.

[85] F. Hou, L. Wen, C. Peng, and J. Guo, "Identification of marine traditional Chinese medicine dried seahorses in the traditional Chinese medicine market using DNA barcoding," Mitochondrial DNA Part A: DNA Mapping Sequencing Analysis, vol. 2016, pp. 1–6, 2016.

[86] Y.-H. Zhang, G. Qin, H.-X. Zhang, X. Wang, and Q. Lin, "DNA barcoding reflects the diversity and variety of brooding traits of fish species in the family Syngnathidae along China's coast," Fisheries Research, vol. 185, pp. 137–144, 2017.

[87] L. Gao, Y. Yin, J. Li et al., "Identification of traditional Chinese medicinal pipishef and exclusion of common adulterants by multiplex PCR based on 125 sequences of specific alleles," Mitochondrial DNA Part A: DNA Mapping Sequencing Analysis, vol. 2017, Article ID 1278538, pp. 1–7, 2017.

[88] R. A. Torres, R. B. Feitosa, D. C. Carvalho, M. O. Freitas, M. Hostim-Silva, and B. P. Ferreira, "DNA barcoding approaches for fishing authentication of exploited grouper species including the endangered and legally protected goliath grouper Epinephelus itajara," Scientia Marina, vol. 77, no. 3, pp. 409–418, 2013.

[89] R. S. Rasmussen, M. T. Morrissey, and P. D. N. Hebert, "DNA barcoding of commercially important salmon and trout species (oncorhynchus and salmo) from north america," Journal of Agricultural and Food Chemistry, vol. 57, no. 18, pp. 8379–8385, 2009.

[90] S. Botti and E. Giuffra, "Oligonucleotide indexing of DNA barcodes: identification of tuna and other scombrid species in food products," BMC Biotechnology, vol. 10, article 60, 2010.

[91] T. N. A. Mat Jaafar, M. I. Taylor, S. A. Mohd Nor, M. de Bruyn, and G. R. Carvalho, "DNA barcoding reveals cryptic diversity within commercially exploited indo-malay carangidae (Teleostei: Perciformes)," PLoS ONE, vol. 7, no. 11, Article ID e94923, 2012.

[92] A. M. J. M. Asis, J. K. M. Lacsamana, and M. D. Santos, "Illegal trade of regulated and protected aquatic species in the Philippines detected by DNA barcoding," Mitochondrial DNA, vol. 27, no. 1, pp. 659–666, 2016.

[93] Y. Zeng, Z. Wu, C. Zhang, Z. Meng, Z. Jiang, and J. Zhang, "DNA barcoding of mobulid ray gill rakers for implementing CITES on Elasmobranch in China," Scientific Reports, vol. 6, Article ID 37567, 2016.

[94] D. Steinke, A. M. Bernard, R. L. Horn, P. Hilton, R. Hanner, and M. S. Shivji, "DNA analysis of traded shark fins and mobulid gill plates reveals a high proportion of species of conservation concern," Scientific Reports, vol. 7, no. 1, article ID 9505, 2017.

[95] S.-Y. V. Liu, C.-L. C. Chan, O. Lin, C.-S. Hu, and C. A. Chen, "DNA barcoding of shark meats identify species composition and CITES-listed species from the markets in Taiwan," PLoS ONE, vol. 8, no. 11, Article ID e79373, 2013.

[96] P.-S. Chuang, T.-C. Hung, H.-A. Chang, C.-K. Huang, and J.-C. Shiao, "The species and origin of shark fins in Taiwan's fishing ports, markets, and customs detention: A DNA barcoding analysis," PLoS ONE, vol. 11, no. 1, Article ID e0147290, 2016.

[97] A. T. Fields, D. L. Abercrombie, R. Eng, K. Feldheim, and D. D. Chapman, "A novel mini-DNA barcoding assay to identify processed fins from internationally protected shark species," PLoS ONE, vol. 10, no. 2, Article ID e0114844, 2015.

[98] D. Carde˜nosa, A. Fields, D. Abercrombie, K. Feldheim, S. K. H. Shea, and D. D. Chapman, "A multiplex PCR mini-barcode assay to identify processed shark products in the global trade," PLoS ONE, vol. 12, no. 10, Article ID e0185368, 2017.

[99] M. J. Maya-Soriano, W. V. Holt, and R. E. Lloyd, "Biobanked amphibian samples confirmed to species level using 16S rRNA"
DNA barcodes,” Biopreservation and Biobanking, vol. 10, no. 1, pp. 22–28, 2012.

[100] J. Che, H.-M. Chen, J.-X. Yang et al., “Universal COI primers for DNA barcoding amphibians,” Molecular Ecology Resources, vol. 12, no. 2, pp. 247–258, 2012.

[101] D. Qiu, C. E. Cook, Q. Yue et al., “Species-level identification of the blowfly Chrysomya megacephala and other Diptera in China by DNA barcoding,” Genome, vol. 60, no. 2, pp. 158–168, 2017.

[102] L. Xiang, J. Song, T. Xin et al., “DNA barcoding the commercial Chinese caterpillar fungus,” FEMS Microbiology Letters, vol. 347, no. 2, pp. 156–162, 2013.

[103] K. Y. C. Lam, G. K. L. Chan, G.-Z. Xin et al., “Authentication of Cordyceps sinensis by DNA analyses: Comparison of ITS sequence analysis and RAPD-derived molecular markers,” Molecules, vol. 20, no. 12, pp. 22454–22462, 2015.

[104] Y. Liu, X.-Y. Wang, Z.-T. Gao, J.-P. Han, and L. Xiang, “Detection of Ophiocordyceps sinensis and its common adulterates using species-specific primers,” Frontiers in Microbiology, vol. 8, article 1179, 2017.

[105] E. Ortiz, G. B. Gurrola, E. F. Schwartz, and L. D. Possani, “Scorpion venom components as potential candidates for drug development,” Toxicon, vol. 93, pp. 125–135, 2015.

[106] V. R. Vartak, R. Narasimmalu, P. K. Annam, D. P. Singh, and W. S. Lakra, “DNA barcoding detected improper labelling and supersession of crab food served by restaurants in India,” Journal of the Science of Food and Agriculture, vol. 95, no. 2, pp. 359–366, 2015.

[107] P. Mirzapur, Z. Rashidi, L. Rezakhani, and M. Khazaei, “In vitro inhibitory effect of crab shell extract on human umbilical vein endothelial cell,” In Vitro Cellular & Developmental Biology - Animal, vol. 51, no. 1, pp. 36–41, 2014.

[108] S. Uthicke, M. Byrne, and C. Conand, “Genetic barcoding of commercial Béche-de-mer species (Echinodermata: Holothuroidea),” Molecular Ecology Resources, vol. 10, no. 4, pp. 634–646, 2010.

[109] R. D. Ward, B. H. Holmes, and T. D. O’Hara, “DNA barcoding discriminates echinoderm species,” Molecular Ecology Resources, vol. 8, no. 6, pp. 1202–1211, 2008.

[110] J. Rombke, M. Aira, T. Backeljau et al., “DNA barcoding of earthworms (Eisenia fetida/andreic process) from 28 ecotoxicological test laboratories,” Applied Soil Ecology, no. 104, pp. 3–11, 2016.

[111] S.-T. Hsiao, S.-C. Chuang, K.-S. Chen, P.-H. Ho, C.-L. Wu, and C. A. Chen, “DNA barcoding reveals that the common cupped oyster in Taiwan is the Portuguese oyster Crassostrea angulata (Ostreoida; Ostreidae), not C. gigas,” Scientific Reports, vol. 6, Article ID 34057, 2016.

[112] J. Liu, Q. Li, L. Kong, H. Yu, and X. Zheng, “Identifying the true oysters (Bivalvia: Ostreidae) with mitochondrial phylogeny and distance-based DNA barcoding,” Molecular Ecology Resources, vol. 11, no. 5, pp. 820–830, 2011.

[113] K. Uda, Y. Komeda, T. Fujita et al., “Complete mitochondrial genomes of the Japanese pink coral (Corallium clathrus) and the Mediterranean red coral (Corallium rubrum): A reevaluation of the phylogeny of the family Corallidae based on molecular data,” Comparative Biochemistry and Physiology - Part D: Genomics and Proteomics, vol. 8, no. 3, pp. 209–219, 2013.

[114] M. L. Coghlan, G. Maker, E. Crighton et al., “Combined DNA, toxicological and heavy metal analyses provides an auditing toolkit to improve.pharmacovigilance of traditional Chinese medicine (TCM),” Scientific Reports, vol. 5, Article ID 17475, 2015.

[115] H. J. De Boer, M. C. Ichim, and S. G. Newmaster, “DNA barcoding and pharmacovigilance of herbal medicines,” Drug Safety, vol. 38, no. 7, pp. 611–620, 2015.

[116] S. K. Lou, K. L. Wong, M. Li, P. P. But, S. K. Tsui, and P. C. Shaw, “An integrated web medicinal materials DNA database: MMDBD (Medicinal Materials DNA Barcode Database),” BMC Genomics, vol. 11, no. 1, article 402, 2010.

[117] F. Austerlitz, O. David, B. Schaeffer et al., “DNA barcode analysis: a comparison of phylogenetic and statistical classification methods,” BMC Bioinformatics, vol. 10, no. 14, article 1471, pp. 1–13, 2009.

[118] S. F. Altschul, T. L. Madden, A. A. Schäffer et al., “Gapped BLAST and PSI-BLAST: a new generation of protein database search programs,” Nucleic Acids Research, vol. 25, no. 17, pp. 3389–3402, 1997.

[119] R. P. Kelly, I. N. Sarkar, D. J. Eernisse, and R. DeSalle, “DNA barcoding using chitons (genus Mopalia): Barcoding,” Molecular Ecology Resources (Formerly known as Molecular Ecology Notes), vol. 7, no. 2, pp. 177–183, 2007.

[120] N. Saitou and M. Nei, “The neighbor-joining method: a new method for reconstructing phylogenetic trees,” Molecular Biology and Evolution, vol. 4, no. 4, pp. 406–425, 1987.

[121] M. Elias, R. I. Hill, K. R. Willmott et al., “Limited performance of DNA barcoding in a diverse community of tropical butterflies,” Proceedings of the Royal Society B Biological Science, vol. 274, no. 1627, pp. 2881–2889, 2007.

[122] R. R. N. Alves, W. M. S. Souto, and R. R. D. Barboza, “Primates in traditional folk medicine: a world overview,” Mammal Review, vol. 40, no. 2, pp. 155–180, 2010.

[123] X. Wang, G. Xu, C. Liu et al., “Development of deft amplification refractory mutation sequencing system (ARMS) for discriminating Pilos antler based on a short cytochrome b (Cytb) gene,” Mitochondrial DNA, 2014.

[124] R. R. Da Nóbrega Alves, W. L. Da Silva Vieira, and G. G. Santana, “Reptiles used in traditional folk medicine: conservation implications,” Biodiversity and Conservation, vol. 17, no. 8, pp. 2037–2049, 2008.

[125] A. M. S. Mayer, A. D. Rodriguez, O. Taglialetela-Scafati, and N. Fusetani, “Marine pharmacology in 2012–2013: Marine compounds with antibacterial, anti-diabetic, anti-fungal, anti-inflammatory, antiprotozoal, antituberculosis, and antiviral activities: affecting the immune and nervous systems, and other miscellaneous mechanisms of action,” Marine Drugs, vol. 15, no. 9, article 273, 2017.

[126] E. Safari, Z. M. Hassan, and S. M. Moazzeni, “Shark cartilage 14kDa protein as a dendritic cells activator,” Immunopharmacology and Immunotoxicology, vol. 37, no. 2, pp. 165–170, 2015.

[127] Q. Yang, X. Zhou, M. Zhang et al., “Angel of human health: current research updates in toad medicine,” American Journal of Translational Research, vol. 7, no. 1, pp. 1–14, 2015.

[128] J. R. Vallejo and J. A. González, “Amphibians in Spanish popular medicine and the pharmacopoeia of Pliny and Dioscorides,” Historia, Ciencias, Saúde - Manguinhos, vol. 22, no. 4, pp. 1283–1319, 2015.

[129] A. D. Garg, R. V. Hippiarg, and A. N. Ganghare, “Toad secretions: Potent source of pharmacologically and therapeutically significant compounds,” The Internet Journal of Pharma- cology, vol. 5, no. 2, p. 17, 2008.
[130] V. B. Meyer-Rochow, “Therapeutic arthropods and other, largely terrestrial, folk-medicinally important invertebrates: a comparative survey and review,” *Journal of Ethnobiology and Ethnomedicine*, vol. 13, no. 1, article 9, 2017.

[131] C. L. Schoch and K. A. Seifert, “Reply to Kiss: Internal transcribed spacer (ITS) remains the best candidate as a universal DNA barcode marker for Fungi despite imperfections,” *Proceedings of the National Academy of Sciences of the United States of America*, vol. 109, no. 27, pp. 6241–6246, 2012.

[132] Y. Guo, Y. Ding, F. Xu et al., “Systems pharmacology-based drug discovery for marine resources: An example using sea cucumber (Holothurians),” *Journal of Ethnopharmacology*, vol. 165, pp. 61–72, 2015.

[133] E. L. Cooper, M. Balamurugan, C.-Y. Huang et al., “Earthworms dulong: Ancient, inexpensive, noncontroversial models may help clarify approaches to integrated medicine emphasizing neuroimmune systems,” *Evidence-Based Complementary and Alternative Medicine*, vol. 2012, Article ID 164152, 11 pages, 2012.

[134] K. Benkendorff, “Molluscan biological and chemical diversity: Secondary metabolites and medicinal resources produced by marine molluscs,” *Biological Reviews*, vol. 85, no. 4, pp. 757–775, 2010.

[135] E. L. Cooper, K. Hirasayashi, K. B. Strychar, and P. W. Sammarco, “Corals and their potential applications to integrative medicine,” *Evidence-Based Complementary and Alternative Medicine*, vol. 2014, Article ID 184959, 9 pages, 2014.

[136] J. Hawkins, N. De Vere, A. Griffith et al., “Using DNA metabarcoding to identify the floral composition of honey: A new tool for investigating honey bee foraging preferences,” *PLoS ONE*, vol. 10, no. 8, Article ID e0134735, 2015.

[137] H. Muhammad Tahir and S. Akhtar, “Services of DNA barcoding in different fields,” *Mitochondrial DNA Part A: DNA Mapping, Sequencing, and Analysis*, vol. 27, no. 6, pp. 4463–4474, 2016.

[138] R. Vilgalys, “Taxonomic misidentification in public DNA databases,” *New Phytologist*, vol. 160, no. 1, pp. 4–5, 2003.

[139] R. A. Collins and R. H. Cruickshank, “The seven deadly sins of DNA barcoding,” *Molecular Ecology Resources*, vol. 13, no. 6, pp. 969–975, 2013.