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DNA barcodes for everyday life: Routine authentication of Natural Health Products

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ABSTRACT

The authenticity of Natural Health Products (NHPs) remains an important legal, economic, health and conservation issue. Here, we use DNA sequence information through standardized DNA barcoding for the authentication of 95 NHPs from animal and plant species of varying form and origin, with more extensive sampling for shark and ginseng products. We were able to identify 75% of all NHPs tested, with success rates of up to 83% and 88% in shark and ginseng products, respectively. Standard DNA barcode region of cytochrome *C* oxidase 1 (*COI*) gene and its smaller mini-barcode sequences were used for barcoding of all animal products. Although the *rbcLa*/*matK* core plant barcodes have limitations in robustly distinguishing Korean and American ginseng species, these species can be differentiated by a supplemental barcode from the Internal Transcribed Spacer (*ITS*) of nuclear ribosomal DNA. Interestingly, half of the samples commercially labelled as Korean ginseng were identified as American ginseng. This reveals a need for the authentication of ginseng-containing products. Ultimately, this work showcases the utility of DNA barcodes for the real-world authentication of Natural Health Products.

1. Introduction

The World Health Organization estimates that 70% to 80% of the developed-world population have used alternative medicines (WHO, 2008). In North America, alternative medicines, often termed "Natural Health Products" (NHPs) include vitamins and minerals, homeopathic remedies, herbal remedies, and traditional medicines such as traditional Chinese medicines (Mine & Young, 2009). Natural Health Products are often perceived to be safe due to their natural origin, however, adulterated, counterfeit and low quality products pose serious safety threats to consumers (Mine & Young, 2009; WHO, 2008). This expanding market presence demands tighter regulations for product safety, and these regulations often require authentication at the raw and finished product stage. Nonetheless, authentication becomes complicated because the original identifying characteristics (e.g. morphological characters) are absent in the majority of NHPs. These products are mainly in forms such as capsules and tablets, or are dried parts of a whole specimen (as in plant roots or shark fins). Reliable species authentication methods are not only critical for the enforcement of regulations, and to avoid adverse health and economic outcomes, but they are also important to reduce the negative environmental effects associated with purchasing protected species marketed as unprotected species.

Molecular methods for the identification of NHPs are being increasingly investigated (Sucher & Carles, 2008; Coghlan et al., 2012). Among different techniques, DNA barcoding has been recognized as a robust, rapid, cost-effective and broadly applicable approach for species identification. DNA barcoding employs sequence variation within a short, standardized region of the genome – a "barcode" – to provide accurate identification at the species-level (Hajibabaei, Singer, Hebert, & Hickey, 2007; Hebert, Cywinska, Ball, & deWaard, 2003). A 658-bp fragment of mitochondrial cytochrome *C* oxidase 1 (*COI*) has been designated as DNA barcode for animals (Hebert, Cywinska, Ball, & deWaard, 2003). For plants, a two-locus chloroplast barcode using *rbcLa* – ribulose-1,5-bisphosphate carboxylase – and *matK* – maturase K genes has recently gained support (Hollingsworth et al., 2009). The Internal Transcribed Spacer (*ITS*) region and chloroplast spacer *trnh-psbA* have also been proposed as DNA barcodes for plants (Chen et al., 2010).

Here, we apply DNA barcoding for a real-world, routine authentication of commonly used plant and animal NHPs acquired directly from commercial markets in North America to assess the extent of misrepresentation and substitution occurring on this market. In particular, we focused our sampling on products containing shark and ginseng, two commonly used NHPs. Shark fins, used to make shark fin soup, are considered an East Asian delicacy and are one of the most expensive seafood products in the world (Fong & Anderson, 2002). Ginseng root is used for its various medicinal properties – in

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particular, as an antioxidant, and an aphrodisiac – and is popular globally (Nocerino, Amato, & Izzo, 2000).

2. Materials and methods

2.1. Specimens

Ninety-five plant and animal products were purchased from various commercial sources, including pharmacies and markets in Toronto, Canada and New York, U.S.A. Samples included capsules, tablets, roots, carved roots, extracts, teas, and dried and shredded products. Of the twenty-five animal products, 12 were shark products, including 8 dried/shredded shark fins, shark cartilage and shark liver oil. Thirteen other products were dried fish, sea cucumber, sea horse, sea scallop, sea dragon, and deer antler and tendon. In addition, samples containing sheep placenta and harp seal oil were also gathered. Of the 70 plant products, 41 were ginseng-containing products, and 29 were other medicinal plants, including Echinacea, St. John's Wort, green tea, milk thistle, feverfew, dandelion, and chamomile, in different forms. See Tables 1–3 for details of samples tested.

2.2. DNA barcode analysis

After homogenisation in a FastPrep-24 instrument (MP Biomedicals, Solon, Ohio, USA), DNA was extracted from 25 mg of animal NHPs and 100 mg of plant NHPs using NucleoSpin Tissue Kits (Macherey-Nagel, Bethlehem, PA, USA) for animals and plants, respectively. For some specimens, multiple samples were taken and barcoded (AP001, AP003, AP012 and AP015). This was because based on their appearance and label, these specimens were expected to contain more than one species. In addition, multiple samples were barcoded for AP010, a cooked specimen with potentially degraded DNA. About 650 bp barcodes of *COI* were amplified in all animal NHPs. When full-barcode recovery failed, 130 bp mini-barcode fragments of *COI* (Meusnier et al., 2008) were amplified. For plant NHPs, core plant barcodes of *rbcL*a and *matK* genes were amplified (Hollingsworth et al., 2009). Additionally, for ginseng samples, *ITS* and chloroplast spacer *trnh-psbA* were amplified as supplemental markers (Chen et al., 2010; Kress & Erickson, 2007).

PCR was performed in 25 μl reactions in a Mastercycler ep gradient S thermalcycler (Eppendorf, Mississauga, ON, Canada). Each PCR reaction contained 2 μl of DNA template (10–100 ng/μl), 17.5 μl molecular biology grade water, 2.5 μl 10× PCR buffer, 1 μl 50× $MgCl_2$ (50 mM), 0.5 μl dNTPs mix (10 mM), 0.5 μl of each forward and reverse primers (10 mM), and 0.5 μl of Invitrogen's Platinum *Taq* polymerase (5 U/μl). PCR products were visualised on 1.5% agarose gels. To ascertain that no exogenous DNA had been introduced to the PCR reaction, a negative control reaction was included in all experiments. All negative PCR reactions were hit picked with the same primer set to ensure maximum amplification success for each sample. See Tables 4 and 5 for details including primer names, sequences and PCR reaction conditions.

All amplicons were bi-directionally sequenced on an ABI 3730XL sequencer (Applied Biosystems, Foster City, CA, USA) with standard protocols for sequencing (Hajibabaei et al., 2005; Ratnasingham & Hebert, 2007). Contig assembly was carried out using Codon Code aligner software, version 3.0.3. Identification of unknown samples was conducted using BLAST against Genbank and a local barcode library for selected taxa with a minimum BLAST cut off of 97% identity for a top match. These results were verified by neighbour-joining analysis and evaluating branches leading to specimens tested as compared to sequences of reference species. Nucleotide variation between ginseng species was assessed for all tested markers using iBarcode.org Haplotype map (Singer & Hajibabaei, 2009). Top species matches for each specimen barcoded were compared with the product label. All sequences have been deposited in GenBank (Accession numbers: JN654220 to JN654340). Alignments generated and neighbour-joining trees are available upon request.

Table 1
Commercial animal products used for DNA barcoding authentication.

Sample code	Market label	Form	Barcoding ID	*COI* sequence
AP-001a[a]	Tiger shark bones[b]	Dried	*Sphyrna tiburno*	655 bp
AP-001b[a]		Dried	*Rhizoprionodon lalandii*	655 bp
AP-001c[a]		Dried	*Rhizoprionodon lalandii*	655 bp
AP-002	Shark fin	Dried	*Alopias pelagicus*	130 bp
AP-003a	Shark fin[b]	Dried, shredded	No sequence	No sequence
AP003b[a]		Dried, shredded	*Pangasius hypophthalmus*	655 bp
AP-003c		Dried, shredded	*Galeocerdo cuvier*	655 bp
AP-004	Sea cucumber	Dried	*Holothuria leucospilota*	130 bp
AP-005	Sea horse	Dried	*Hippocampus trimaculatus*	655 bp
AP-006	Shark fin	Dried	*Alopias pelagicus*	655 bp
AP-007	Sea scallops	Dried	No sequence	No sequence
AP-008[a]	Abalone	Dried slice	*Cymbium olla*	130 bp
AP-009	Sea dragon	Dried	No sequence	No sequence
AP-010a	Fish stomach	Cooked and Dried	No sequence	No sequence
AP-010b		Cooked and Dried	Sebastes sp.	130 bp
AP-010c		Cooked and Dried	Sebastes sp.	130 bp
AP-011	Shark fin	Dried	*Alopias pelagicus*	655 bp
AP-012a	Shark fin[b]	Dried, shredded	*Sphyrna zygaena*	655 bp
AP-012b		Dried, shredded	*Alopias pelagicus*	655 bp
AP-012c		Dried, shredded	*Rhizopriondon acutus*	655 bp
AP-013	Sea horse	Dried	*Hippocampus trimaculatus*	655 bp
AP-014	Sea bird	Dried	No sequence	No sequence
AP-015a	Shark fin[b]	Dried, shredded	*Sphyrna lewini*	655 bp
AP-015b		Dried, shredded	*Sphyrna lewini*	655 bp
AP-015c		Dried, shredded	*Sphyrna lewini*	655 bp
AP-016	Shark cartilage	Dried	*Prionace glauca*	130 bp
AP-017	Shark star	Capsule	No sequence	No sequence
AP-018	Deer antler	Dried	*Cervus elaphus*	130 bp
AP-019	Shark liver oil	Capsule	No sequence	No sequence
AP-020	Sheep placenta	Capsule	No sequence	No sequence
AP-021	Harp seal oil	Capsule	No sequence	No sequence
AP-022	Velvet antler	Capsule	*Cervus elaphus*	130 bp
AP-023	Deer tendon	Dried	*Cervus elaphus*	130 bp
AP-024	Shark fin	Dried	*Sphyrna lewini*	655 bp
AP-025	Shark fin	Dried	*Carcharhinus limbatus*	655 bp

[a] Substitution.
[b] Multiple samples barcoded because specimen expected to contain more than one shark species.

3. Results and discussion

3.1. Animal products

DNA barcodes were recovered from 72% of animal NHPs. Eighty-five percent of all shark samples, 60% of fish samples, and 60% of mammalian products (deer, seal and sheep) were successfully identified by DNA barcoding. In specimens with potentially degraded DNA (e.g. AP010),

Table 2
Commercial ginseng products used for DNA barcoding authentication.

Sample code	Market label	Form	Barcoding ID	*rbcLa* ID	*matK* ID	*psbA-trnh* ID	*ITS* ID
GIN-001	Ginseng	Tea	PQ	PQ	NS	PS	NS
GIN-002	American ginseng	Root	PQ	PQ	PS	PS	NS
GIN-003[a]	Korean ginseng	Carved Root	PQ	PQ	PS	PS	PQ
GIN-004	Canadian ginseng	Root	PQ	PQ	PS	PS	PQ
GIN-005	Canadian ginseng	Carved Root	PQ	PQ	PS	NS	NS
GIN-006	Canadian ginseng	Root	PQ	PQ	PS	PS	PQ
GIN-007[a]	Korean ginseng	Root	PQ	PQ	PS	PS	PQ
GIN-008[a]	Red Korean ginseng	Carved Root	PQ	NG	NG	NS	PQ
GIN-009[a]	Korean ginseng	Carved Root	PQ	PQ	PS	PS	NS
GIN-010[a]	Korean ginseng	Tea	PQ	NS	NS	NS	PQ
GIN-011	Canadian ginseng	Root	PQ	PQ	PS	PS	NS
GIN-012	Canadian ginseng	Carved Root	PQ	PQ	PS	PS	NS
GIN-013	Canadian ginseng	Carved Root	PQ	PQ	PS	PS	NS
GIN-014	Panax red Korean	Capsule	PG	PG	PS	PS	PG
GIN-015	Canadian ginseng	Root	PQ	PQ	PS	PS	NS
GIN-016[a]	Korean ginseng	Root	PQ	PQ	NG	PS	PQ
GIN-017	Canadian ginseng	Carved Root	PQ	PQ	PS	PS	NS
GIN-018[a]	Korean ginseng	Root	PQ	PQ	PS	PS	PQ
GIN-019	Chinese ginseng	Root	PG	PG	PS	PS	PG
GIN-020	Korean red	Carved Root	NS	NS	NS	NS	NS
GIN-021	Canadian ginseng	Root pieces	PQ	PQ	PS	PS	PQ
GIN-022	Panax ginseng	Capsule	NS	NS	NS	NS	NS
GIN-023	Chinese red panax	Liquid	NS	NS	NS	NS	NS
GIN-024	Sand ginseng	Root	NG	NG	NG	NS	NG
GIN-025	American ginseng	Tea	PQ	PQ	PS	NS	PQ
GIN-026	Korean ginseng	Drink /root	PG	NG	NS	NS	PG
NY2	Korean ginseng	Tea	PS	NS	PS	NS	NS
NY3	American ginseng	Root pieces	PQ	PQ	NS	PS	PQ
NY4[a]	Korean ginseng	Root pieces	PQ	PQ	PS	PS	NS
NY5[a]	Korean ginseng	Root slices	PQ	PQ	NS	PS	PQ
NY6[a]	Korean ginseng	Root slices	PQ	PQ	NS	PS	PQ
NY7	American ginseng	Root slices	NS	NS	NS	NS	NS
NY8	American ginseng	Root pieces	PQ	PQ	PS	PS	PQ
NY9	American ginseng	Root pieces	PQ	PQ	PS	PS	PQ
NY10	Korean ginseng	Tea	PG	PG	PS	PS	PG
MDP-018	Korean ginseng	Caplet	NS	NS	NS	NS	NS
MDP-019[a]	Korean ginseng	Caplet	PQ	NS	NS	NS	PQ
MDP-020	Korean ginseng	Tablet	NG	NG	NS	NS	NS
MDP-021	Korean ginseng	Capsule	NG	NG	NS	NS	NS
MDP-022	American ginseng	Caplet	PQ	PS	PQ	NS	NS
MDP-023	American ginseng	Capsule	PQ	PS	PQ	NS	NS

Panax quinquefolius (PQ), *Panax ginseng* (PG), No Sequence (NS), Non ginseng (NG), Panax. sp. (PS)
[a] Substitution of American ginseng for Korean ginseng.

full-length DNA barcodes (~650 bp) could not be sequenced and mini-barcodes of 130 bp were sequenced instead. Out of the 26 sequences obtained for animal products, 65% were full-length barcodes and 35% were 130 bp mini-barcodes. Interestingly, in a product composed of shredded shark fins (AP012), we obtained the DNA barcodes of three different species by sampling and analysing three different regions on the specimen — a Pelagic Thresher (*Alopias pelagicus*), a Common Hammerhead (*Sphyrna zygaena*) and a Milk Shark (*Rhizopriondon acutus*). Similarly, two other samples produced mixed species results when multiple regions on the specimen were analysed (Fig. 1).

Of the 26 animal *COI* barcodes obtained, 21 (81%) correctly matched their commercial label. In one case (AP001), a specimen labelled tiger shark "fin bones" did not contain Tiger shark, but instead contained Bonnethead shark (*Sphryna tiburno*) and Brazillian Sharpnose shark (*Rhizopriondon lalandii*). According to the Food and Agriculture Organization of the United Nations, Tiger shark fins may be more preferred than those of other species due to a number of factors, including a high percentage of fin needles, as well as needle size, appearance and texture (Vannuccini, 1999). Thus, due to adulteration, the package of dried "fin bones" may be worth less than its retail price.

Another potentially adulterated case involved a product (AP003) labelled as a shredded shark fin. Multiple analyses of this sample, recovered Tiger shark (*Galeocerdo cuvier*) as well as a catfish species *Pangasius hypophthalamus*, commonly known in the fish food market as "Iridescent shark". Due to adulteration with catfish, AP003 is not composed of purely shark and is worth less than its commercial value. This adulteration could have been knowingly introduced to reduce manufacturing costs. In addition, *Pangasius hypothalmalus*' common name is "Iridescent shark", although it is in fact a catfish, which could cause confusion for a non-expert (Fig. 1).

Finally, AP008 was commercially labelled as abalone, (genus *Haliotis*), however, the specimen was suspected to be substituted with *Cymbium olla*, another marine gastropod. As world demand for abalone currently exceeds supply, abalone has become a luxury product (Viana, 2002). This substitution could also have been a case of taxonomic misidentification, as these species may be difficult for a non-expert to differentiate.

Several of the shark species identified are listed on the International Union for the Conservation of Nature's "Red List" as in need of conservation. Among these were two cases of *Sphyrna lewini* (AP015 and AP024), the Scalloped Hammerhead, which is ranked as endangered. Species listed as vulnerable included *Alopias pelagicus*, the Pelagic Thresher (AP002, AP006, AP011 and AP012), and *Sphyrna zygaena* (AP012), the Common Hammerhead. The DNA barcode analysis we performed on shark samples was readily capable of identifying species of sharks used in the NHPs. This illuminates the fact that

Table 3
Non-ginseng medicinal plants used for DNA barcoding authentication.

Sample code	Market label	Dosage form	Barcoding ID	*rbcLa* ID	*matK* ID
MDP-001	Black cohosh	Capsule	*Actaea asiatica*	*Actaea asiatica*	NS
MDP-002	Black cohosh	Caplet	NS	NS	NS
MDP-003	Black cohosh	Tablet	NS	NS	NS
MDP-004	Black cohosh	Capsule	NS	NS	NS
MDP-005	Green tea extract	Capsule	*Oryza sativa*	*Oryza sativa*	NS
MDP-006	Green tea extract	Capsule	*Glycine max*	*Glycine max*	NS
MDP-007	Green tea extract	Capsule	*Oryza sativa*	*Oryza sativa*	NS
MDP-008	Green tea extract	Capsule	NS	NS	NS
MDP-009	Green tea extract	Capsule	NS	NS	NS
MDP-010	*Echinacea purpurea*	Capsule	NS	NS	NS
MDP-011	*Echinacea purpurea*	Capsule	NS	NS	NS
MDP-012	*Echinacea purpurea*	Capsule	NS	NS	NS
MDP-013	*Echinacea purpurea*	Capsule	*Asteraceae* sp.	*Asteraceae* sp.	NS
MDP-014	*Echinacea purpurea*	Capsule	*Juglandaceae* sp.	*Juglandaceae* sp.	NS
MDP-015	St. John's wort	Caplet	NS	NS	NS
MDP-016	St. John's wort	Capsule	NS	NS	NS
MDP-017	St. John's wort	Caplet	NS	NS	NS
MDP-024	Milk thistle	Caplet	NS	NR	NS
MDP-025	Milk thistle	Capsule	*Asteraceae* sp.	NR	*Asteraceae* sp.
MDP-026	Milk thistle	Capsule	NS	NR	NS
MDP-027	Milk thistle	Capsule	NS	NR	NS
MDP-028	Feverfew	Capsule	*Asteraceae* sp.	*Asteraceae* sp.	NS
MDP-029	Feverfew	Capsule	*Asteraceae* sp.	*Asteraceae* sp.	NS
MDP-030	Dandelion	Capsule	NS	NS	NS
MDP-031	Dandelion	Capsule	*Taraxacum officinale*sp.	*Taraxacum officinale*	NS
MDP-032	Green tea extract	Tea	*Camellia sinensis*	*Camellia sinensis*	*Camellia sinensis*
MDP-033	Green tea extract	Tea	*Camellia sinensis*	*Camellia sinensis*	NS
MDP-034	Chamomile	Tea	*Asteraceae* sp.	*Asteraceae* sp.	*Asteraceae* sp.
MDP-035	Dandelion	Tea	*Asteraceae* sp.	*Asteraceae* sp.	NS

No Sequence (NS), No reference (NR), *Substitution.

beyond identifying mislabelled products, DNA barcoding of NHPs could help to provide a checkpoint for conservation organisations investigating species exploitation.

3.2. Plant products

Barcode sequences were recovered from 75% of plant NHPs. Success rates for ginseng and non-ginseng products were 88% and 59%, respectively. Sequencing success for each ginseng marker for *ITS*, *psbA-trnh*, *matK*, *rbcLa* were 51%, 59%, 66% and 80%, respectively. For other medicinal plants, *rbcLa* had a sequencing success rate of 58% while *matK* had a sequencing success rate of 10% (Tables 2 and 3).

All sequenced ginseng products labelled "American ginseng (*Panax quinquefolius*)" were the correct species. However, 50% of sequenced ginseng products labelled "Korean ginseng (*Panax ginseng*)" were American ginseng (*P. quinquefolius*) (Fig. 2). We also recovered two non-ginseng barcodes from two ginseng products, MDP020 and MDP021. DNA-based identification of these two commonly used species of ginseng (*P. quinquefolius* and *P. ginseng*) is complicated because of the lack of sufficient nucleotide variation in core plant DNA barcode markers *rbcLa* and *matK* (Chen et al., 2010). We only observed one nucleotide variation at *rbcLa* in the samples examined. Interestingly, the presumably more variable spacer region, *trnH-psbA* shows no variation between the two species. However, the sequenced region of nuclear *ITS* shows four nucleotide differences between American and Korean ginseng, enabling robust species identification. Our observations reinforce the importance of supplementing core-barcodes with markers that can provide robust species-level resolution, especially in cases where species identification is linked to the authentication of socioeconomically important specimens (Li et al., 2011). Of the 14 non-ginseng samples that yielded a DNA sequence, two are suspected to be adulterated. In one case, using *rbcLa*, a specimen (MDP014) commercially labelled as Echinacea (*Echinacea purpurea*), closely matched species from the walnut family (Juglandaceae). It is unclear how such a substitution could occur, but this situation could be particularly dangerous for a consumer with nut allergies. In another case, MDP001, *Acetea asiatica* was detected in a product labelled "Black Cohosh" (*Actaea racemosa*). Health Canada reports that products not containing authentic Black Cohosh may be linked with adverse liver reactions (Painter, Perwaiz, & Murty, 2010).

DNA barcodes obtained from three capsules containing "Green Tea Extract" (*Camellia sinensis*) (MDP005, MDP006 and MDP007), and two capsules of Korean ginseng (MDP020 and MDP021), failed to match their commercial label. For example, MDP005 and MDP007 matched rice (*Oryza sativa*), while MDP006 matched soybean (*Glycine max*). These cases are most likely linked to capsule additives, derived from plant origin. Plant products and other potential PCR inhibitors – for

Table 4
PCR amplification and sequencing primers used for DNA barcoding of commercial Natural Health Products.

Sample	Barcoding marker	Primer code	Sequence (5′–3′)	Reference
Medicinal plants	*rbcLa*	*rbcLa* _F	ATGTCACCACAAACAGAGACTAAAGC	(Levin et al., 2003)
		rbcLa _R	GTAAAATCAAGTCCACCRCG	
	matK	*matK_F*	ACCCAGTCCATCTGGAAATCTTGGTTC	(Fofana, Harvengt, Baudoin, & du Jardin, 1997)
		*matK*_R	CGTACAGTACTTTTGTGTTTACGAG	
	psbA-trnh	*psbA3*_F	GTTATGCATGAACGTAATGCTC	(Kress & Erickson, 2007)
		*trnHf_05*_R	CGCGCATGGTGGATTCACAATCC	
	ITS	*ITS*_8F	AACAAGGTTTCCGTAGGTGA	(Wen & Zimmer, 1996)
		*ITS*_10F	CGAACACGTTACAATACCG	
		*ITS*_9R	TATGCTTAAAYTCAGCGGGT	
		*ITS*_11R	ACCACTTGTCGTGACGTCC	
Animal products	*COI (658 bp)*	VF2_F	CAACCAACCACAAAGACATTGGCAC	(Ivanova, Zemlak, Hanner, & Hebert, 2007)
		FishF2_F	CGACTAATCATAAAGATATCGGCAC	
		FishR2_R	ACTTCAGGGTGACCGAAGAATCAGAA	
		FR1d_R	ACCTCAGGGTGTCCGAARAAYCARAA	
	COI (130 bp)	Uni-Mbar_F	TCCACTAATGCTAARGATATTGGTAC	(Meusnier et al., 2008)
		Uni-Mbar_R	GAAAATCATAATGAAGGCATGAGC	

Table 5
PCR amplification regime used for each barcoding marker.

Gene	Initial denaturation	Repeat 35 cycles			Final extension
		Denaturation	Annealing	Extension	
rbcLa	95 °C/4 min	94 °C/30 s	55 °C/30 s	72 °C/1 min	72 °C/10 min
matK	94 °C/5 min	94 °C/30 s	52 °C/20 s	72 °C/50 s	72 °C/5 min
psbA-trnh	95 °C/4 min	94 °C/30 s	55 °C/1 min	72 °C/30 s	72 °C/10 min
ITS	95 °C/4 min	94 °C/10 s	50 °C/5 s	72 °C/1 min	72 °C/4 min
COI (658 bp)	95 °C/2 min	94 °C/30 s	52 °C/40 s	72 °C/1 min	72 °C/10 min
COI (130 bp)	95 °C/4 min	94 °C/30 s	46 °C/60 s	72 °C/30 s	72 °C/5 min

example, microcrystalline cellulose, and gelatin – are often used in the preparation of capsulated products and may be contained in the sample (Foster et al., 2004). This may produce a mixed signal, contributing to a rather high percentage of failed sequencing reactions in capsulated products. For example, of the five animal capsules attempted, only one capsule, AP022 could be sequenced. Alternatively, some products or capsule contents (e.g. GIN023, AP017, AP019, and AP021) contain a gel or liquid material, which may be in the form of a chemical extract. To alleviate mixed sequencing signals, it is possible to use parallelized next-generation sequencing (such as 454-Roche or Illumina Miseq) in an environmental barcoding approach (Hajibabaei, Shokralla, Zhou, Singer, & Baird, 2011; Shokralla, Spall, Gibson, & Hajibabaei, 2012). Aside from the presence of PCR inhibitors, the amplification failure in some samples, especially in *matK* gene, can also be explained by the fact that primer sets used in this analysis may not be suitable for amplification of all species. In addition, degradation at primer binding sites and varied gene copy number may also contribute to differential amplification success of selected genes in samples with potentially degraded DNA. To overcome the aforementioned amplification failure, multiple markers and/or multiple primer sets as well as different concentrations of template DNA should be used for DNA barcode authentication of plant NHPs.

Although mislabelling can occur due to economic reasons, or misunderstanding, mislabelling ultimately stems from a lack of regulation of Natural Health Products. Poor regulation of NHPs may inevitably be traced back to government legislation and its enforcement. For example, in Canada, a distinct regulatory framework for Natural Health Products did not come into full effect until 2004 (Mine & Young, 2009). Since Natural Health Product Legislation was put into place, there has been a backlog of product licence applications to be processed. At some times, this has led thousands of products to exist on the market without full product licenses (Health Canada, 2012). Moreover, although regulations for the safety, efficacy and quality of Natural Health Products exist in North America, the enforcement of these regulations is particularly difficult at the level of informal markets. Our results indicate that mislabelling in products from informal markets is much more common than in products purchased from large, popular, retail locations. All of the mislabelled

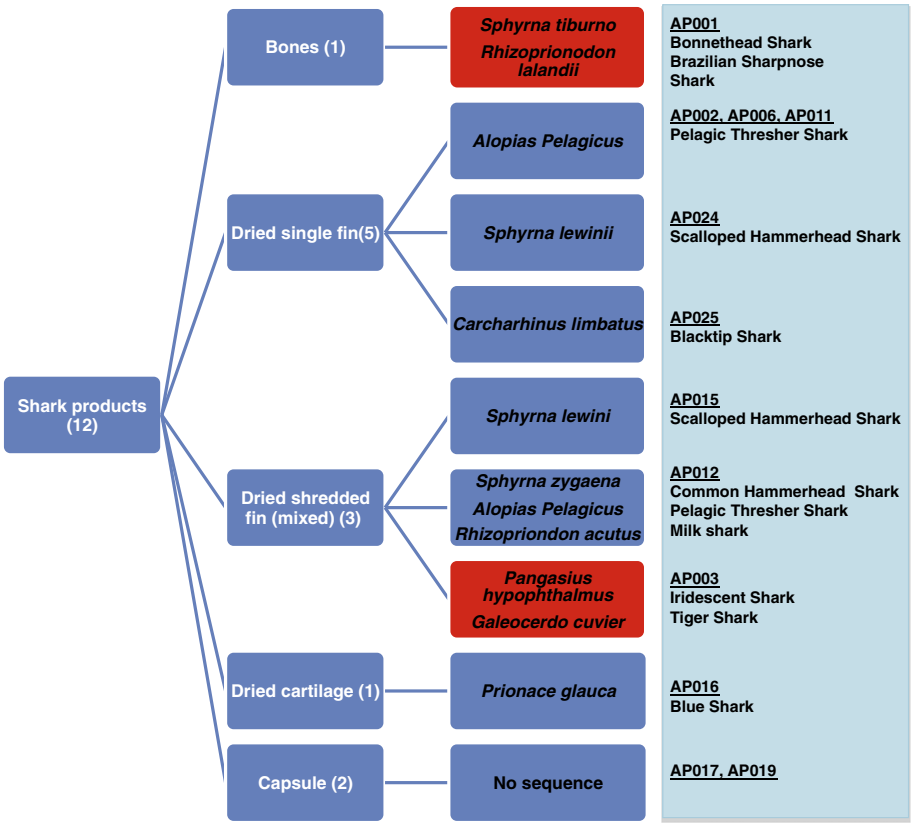

Fig. 1. Flowchart comparing commercial label and DNA barcode identification of shark products.

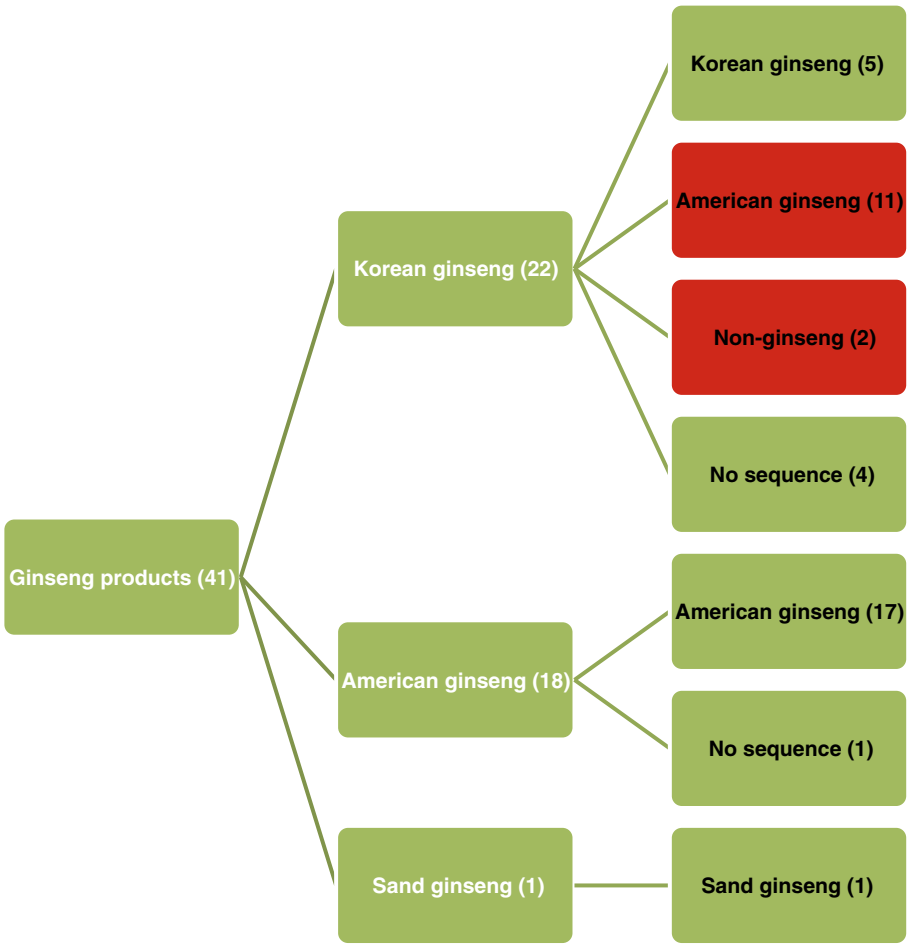

Fig. 2. Flowchart comparing commercial label and DNA barcode identification of ginseng products.

shark fin products and 91% of the mislabelled ginseng products included in our study were purchased at informal markets.

4. Conclusions

The current study strongly indicates that regulatory monitoring for Natural Health Products is insufficient. Product mislabelling can be prevented if more checkpoints are in place to ensure that dishonest practices or accidental substitutions in the marketing of Natural Health Products do not occur. The utility of DNA barcoding for food regulation has already caught the interest of regulatory agencies such as the U.S. Food and Drug Administration (FDA), and has been increasingly investigated (Wong & Hanner, 2008). Our analysis further reinforces the effectiveness of DNA barcoding as an authentication tool for Natural Health Products with widespread applicability. Our work further demonstrates that DNA barcoding will not only identify the substitution and adulteration of NHPs and can aid their associated economic, legal, health and environmental implications, but the approach is simple to use. For instance, the co-authors of this study (LJW, SB, SE) were researchers undergoing their first-time exposure to molecular techniques; however, they were able to learn and successfully perform various tasks from sampling to interpretation of results. This characteristic is essential in the NHP regulatory environment, where non-experts must examine different plant or animal products. Ultimately, DNA barcoding provides a simple and efficient method for accurate species identification of Natural Health products and can play a key role in developing a more robust protocol for their regulation.

Author Contributions

Conceived and designed the experiments: M.H. and S.S. Performed the experiments and analysed the data: L.W., S.B., S. E., and S.S. Wrote the paper: L.W., S.S., M.H. and J.S.

Competing financial interests

The authors declare no competing financial interests.

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