Current Trends in Biotechnology and Pharmacy
Vol.10 (4) 384-402 October 2016, ISSN 0973-8916 (Print), 2230-7303 (Online)

DNA Barcoding as an Authentication Tool for Food and Agricultural Commodities

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Abstract

Adulteration detection and food authenticity testing are important for value assessment and to ensure consumer satisfaction.New generation DNA based adulteration detection methods such as DNA barcoding is becoming an important tool either as to complement the existing physical,sensory,or biochemical analytical methods or as a stand-alone tool due to its cost effectiveness, high through put,sensitivity and reliability. DNA barcoding is a relatively simple technique that is based on the sequence variation in short nucleotide regions called barcodes that enables species identification and commodity authentication. This review provides an insight into the emerging potential of DNA barcoding for authentication of food and agricultural commodities like seafood, spices, medicinal plants, tea etc.

Keywords: Adulterants, Barcoding loci,DNA markers, Food safety, Value assessment.

Introduction

Food adulteration is an issue of concern having a major social, economic and environmental impact. Adulteration may be defined as mixing or substituting the original material with other spurious, inferior, defective, spoiled, useless parts of the same or different plant or harmful substances, synthetic chemicals which do not conform with the official standards. Adulteration can be in two ways - direct/ intentional adulteration and indirect/unintentional adulteration. Direct/intentional adulteration includes practices of substitution partially or fully with inferior materials owing to their morphological resemblance or chemicals or inert materials in order to attain economical gain. Unintentional adulteration results mainly due to the absence of a proper evaluation method (1), negligence and clerical errors (2) etc.

Adulteration detection and authenticity testing of food and agricultural commodities of plant origin including cereals, legumes, beverages, olive oil, fruit products, spices and traded medicinal plant materials are important for value assessment, to check unfair competition and of all to ensure consumer protection against fraudulent practices commonly observed in unscrupulous trade. Also, deceitful adulteration of these products containing, undeclared constituents may cause intoxication or problems such as allergy in sensitive individuals (3,4, 5).

Detection of adulteration and determination of authenticity and quality of foods and food ingredients, including spices are major challenges for the agricultural and food industry, issues that have become increasingly important in recent years (6). Regulatory agencies, food processors and consumers are all interested in detecting adulterants or authenticating raw materials of food products in order to satisfy food quality and safety requirements (7). With globalisation of trade, the role of standards and conformity assessment are of paramount importance because non-tariff agreements such as Sanitary and Phytosanitary (SPS) and Pre

shipment Inspection (PSI) agreements insist that the product(s) is safe, free from adulterants and has the desired quality.

International organisations like the International Organisation for Standardisation (ISO), American Spice Trade Association (ASTA),US Food and Drug Administration (FDA), The Food Safety and Standards Authority, India (FSSAI) etc. impose strict regulations on the quality of food products, spices and herbs imported and exported. Globalisation of food trade requires the development of integrated approaches, such as traceability of origin, quality and authenticity to ensure food safety and quality (8). In the post-WTO era, importing countries as well as the consumers pay more and more attention to food quality, demanding clearer product traceability as well as the use of detailed and accurate product labels.

Numerous techniques have been developed to counter adulteration owing to the increased consumer awareness of food safety and quality control. Adulteration determination is mainly accomplished by comparing measured analytical data with a proper reference set of historical or control data (9). Three strategies are employed for demonstrating admixture in agricultural commodities: demonstrating the presence of a foreign substance in the commodity, demonstrating that a component is present at a concentration which deviates significantly from its normal level and checking the chemical profile of the sample of which the first strategy is considered as the simplest and efficient (3). Authentication tools utilised vary widely depending on the commodity and processes involved ranging from structural evaluation using physical methods, chemical profiling-based analytical methods and the most advanced biotechnological approaches (10).

Physical methods involved in the authentication are macroscopic and microscopic structural evaluation and other parameters such as solubility, bulk density, texture etc. (3). Analytical methods used for food authenticity testing involve chromatographic techniques like High performance liquid chromatography (HPLC), Thin layer chromatography (TLC), Gas chromatography (GC), Spectroscopic methods like UV spectroscopy, Raman Spectroscopy and its variants, Nuclear magnetic resonance spectroscopy (NMR), Mass spectroscopy, Capillary electrophoresis, Hyphenated techniques that differentiate the samples based on the variation in their chemical profile (11-20). Though physical and chemical methods are amenable for food authentication, in certain instances they fail to give correct results (21). The usage of physical methods is often limited due to their time consuming procedure and need for a skilled expertise. The requirement of an expensive standard and the non-availability of standards for certain botanicals restrict the use of analytical methods in food authentication (22). Molecular methods can compensate these limitations and are dominant over the physical and chemical approaches due to its accuracy, effectiveness and non-dependence on the physical form of the sample (whole or powdered), age, environmental factors, storage and processing conditions, especially for the biological adulterants (23,24).

Molecular methods involve the amplication of one or more regions of genomic DNA using polymerase chain reaction and have a great potential in food authentication due to its sensitivity, rapidity, specificity and simplicity (6). The different PCR based methods used for food authentication and traceability are random amplified polymorphic DNA (RAPD) (25,26), arbitrarily primed PCR (AP-PCR) (27), DNA amplification fingerprinting (DAF) (28), inter-simple sequence repeat (ISSR) (29), directed amplification of minisatellite-region DNA (DAMD) (30), sequence characterised amplified regions (SCAR) (31,32), amplification refractory mutation system (ARMS) (33), simple sequence repeat (SSR) analysis (34,35), species specific PCR (36), single nucleotide polymorphism (SNP) (37) and real time PCR (38).

Apart from these techniques, DNA barcoding, a recently evolved molecular marker

Vol.10 (4) 384-402 October 2016, ISSN 0973-8916 (Print), 2230-7303 (Online)

is gaining acceptance and dominance, as a tool for food authentication and traceability, over the other DNA based methods due to their universality and reliability since last 6 -7 years (Fig. 1).

DNA Barcoding : The concept of DNA Barcoding, proposed and developed by Dr. Paul Hebert, a Canadian Biologist, in animals for species identification, is based on the sequence variation in short nucleotide stretches called "barcodes" between species (39). These barcode regions could act as a species recognition tag by comparing it with the sequences present in a reference database containing sequences of the standardized barcode region of almost all the organisms. If an organism fails to match with any sequence in the database, it could be considered as a possible new species (40). An ideal barcode should be easily amplifiable, amenable to sequencing, exhibit higher interspecies variation than intraspecies variation, easily annotated for evaluation of sequence quality and error detection, recoverable from degraded samples (41). A short 648bp region at the 5' end of mitochondrial cox1 (CO1) gene known as the Folmer region, coding for the cytochrome c oxidase subunit served as the standard barcode in animal kingdom (42). The use of the mitochondrial region in barcoding was dominant over the nuclear genome as it did not exhibit gene duplications common in nuclear genome (43).

Increasing work in the arena of DNA barcoding paved way for the formation of two International collaborations, The Consortium for the Barcode of Life (CBOL) and International Barcode of Life (iBOL), for the progression of DNA Barcoding. CBOL is an organization consisting of more than 200 members representing 50 countries estabilished in May 2004 with the support from Alfred P Sloan Foundation, USA to promote DNA barcoding as a global tool for species identification by compiling sequences in a reference DNA library. iBOL, comprising members from 25 nations was set up in 2014 at Guelph, Ontario with an objective to barcode 5 million specimens and 500,000 estimated species present on earth by 2015 (44). The huge data generated by these two organizations initiated the development of Barcode of Life Database (BOLD), a work bench for the acquisition, storage, analysis and publication of DNA barcode data maintained by the University of Guelph, Ontario, Canada (45).

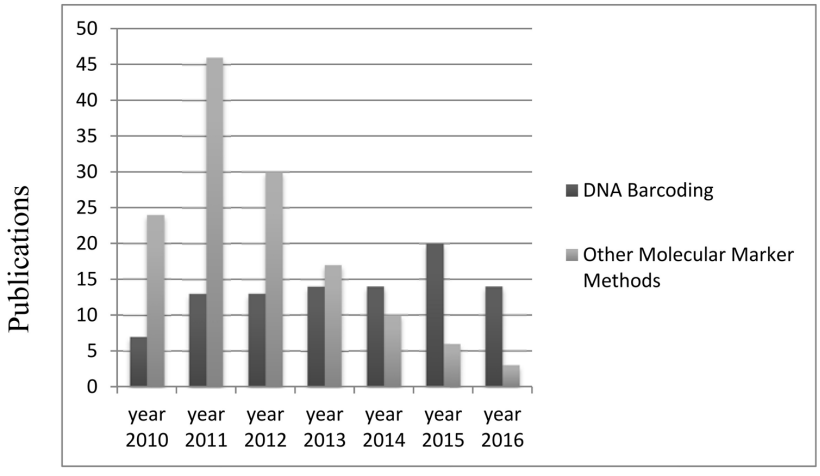

Fig. 1. Publications on DNA barcoding vs.other DNA based methods in food and agricultural commodity authentication.

DNA Barcoding –the Plant Saga : Attempts at plant barcoding using *CO1* gene was a failure due to the low rate of plant species discrimination owing to lack of nucleotide substitutions and high rate of chromosomal rearrangements due to intramolecular recombination in mitochondrial plant genome (46-49). Genome - wide horizontal gene transfer, hybridization and homoplasy also restricted the development of barcoding regions for plants (50). Inspite of these hindrances, regions of chloroplast and nuclear genome were proposed as possible candidates for barcoding in plants. Among the nuclear genomic regions, only the internal transcribed spacer (ITS) of nuclear ribosomal DNA could be used as a barcode owing to lack of universal primers for amplifying the other single copy genes or introns (48) and the technical issues caused by gene duplication in the nuclear genome (51). So focus was shifted to chloroplast genome for barcoding in plants. Regions of chloroplast genome, analogous to mitochondrial genome, sharing its characteristics like conserved gene arrangement, high copy number and availability of universal primers, were proposed as potential candidates as plant barcodes (52). The structural stability, uniparental inheritance and haploid nature of chloroplast genome also facilitated its use as a barcode in plants (53). Internal transcribed spacer of the nuclear ribosomal cistron, some of the coding regions like *rbcL, matK, rpoC1, rpoB* and non-coding regions of the chloroplast genome like *trnH-psbA, atpF-atpH, psbK-psbI, tnnL-trnF* that meet the criteria were proposed as candidate barcodes in plants. In some cases, individual loci failed to serve its purpose. Multi-locus barcode approach was introduced to overcome the same where a phylogenetically conserved easily aligned locus (*rbcL)* is combined with more rapidly evolving variable regions like *matK* or *psbA-trnH* (54). Complementing of these loci will ensure species discrimination and assignment of plants to the correct genus (55).

DNA Barcoding as an Authentication Tool for Food and Agricultural Commodities : DNA barcoding is an efficient marker technique with an important role in certifying food origin, quality of food, safeguarding public health and minimizing food piracy (56, 57). It is based on the analysis of the polymorphic sites in the barcode sequences generated, for the raw materials employed and the food products derived from them (58, 39). These sequences can be compared with the standard sequences deposited in the easily accessible reference databases like GenBank and BOLD to ensure food authenticity and protect consumers from food fraud (58). Increased sensitivity, target DNA sequence diversity, amplification of minute amounts of DNA as in processed products makes DNA barcoding amenable as an authentication tool. Thus, DNA barcoding is widely used for the authentication of medicinal plants and spieces, other agricultural commodities, tea, olive oil, seafood and meat (59-63).

Authentication of Medicinal Plants : The efficacy of herbal medicines mainly depends on the quality of the raw materials used. Unfortunately by default or design many of the herbs mentioned in various pharmacopeia are adulterated taking advantage of the absence of an easy and reliable analytical tool to identify the genuine material from the spurious one. DNA barcoding has now emerged as a very useful approach to authenticate medicinal herbs (Table 1).

Authentication of Spices : Spices are low volume high value commodities traded globally as food flavorant, nutraceuticals,or for medicinal and cosmetic uses. Value added forms of spices like spice powders, crystals, oils and oleoresins. after having lost their morphological diagnostic features are more vulnerable to adulteration than the whole commodity. As in case of medicinal herbs, DNA barcoding based adulteration detection and authentication methods are becoming very popular in traded spices too (Table 2). Adulteration detection can be done at band level or sequence level. In case of band level analysis, the adulterant and genuine product may differ in their amplicon size for a particular gene. The incidence of chilli(*Capsicum annuum* L.) adulteration in traded black pepper (*Piper nigrum*

L.) powder based on the difference in the band size of *trnH-psbA* amplicons was reported (105). *Piper nigrum* samples gave amplicons of size 350bp while adulterated samples (with chilli) gave amplicons of 650bp and 350bp (Fig. 2), respectively for chilli and black pepper.

Authentication of other Agricultural Commodities: Based on the amplification, sequencing success and resolution power, *matK and At103* were proved to be ideal for discrimination of poisonous plants from other useful plants (114).

Canola and saffron oil contamination in olive oil could be detected using DNA barcodes (61). *psbA-trnH* and *matK* primers specific for canola, saffron and olive oil were designed and successfully amplified. These primers were able to detect 5% and above adulteration in olive oil samples. Further confirmation was done by sequencing the amplicons produced and comparing it with the barcodes deposited in reference databases.

A study on barcoding of herbal teas using barcoding loci *rbcL* and *matK* revealed that 35% of the samples generated barcodes for unlisted ingredients on the brand label. *Matricaria recutita* was the most common unlisted ingredient found in seven herbal tea products. Four herbal teas yielded *Camellia sinensis* barcodes although it was not listed on the label. Barcode from a herbal tea sample was similar to *Poa annua* and four products generated barcodes similar to plants of parsley family (115).

DNA barcoding was employed to authenticate the plants used in the preparation of Chinese "cooling beverage",obtained from single or mixture of plants (116). *rbcL, matK, psbA-trnH* and ITS loci were used in the differentiation of Kudidan (*Elephantopus scaber*) from its substituent *Elephantopus tomentosus*; Ludougen (*Pandanus tectorius*) from its adulterant (*Pandanus austrosinensis*); Shepaole (*Rubus reflexus*) from *Rubus parvifolius* and Xiangsizi (*Abrus precatorius*) from *Abrus cantoniensis*. *psbA-trnH, ITS, matK* and *rbcL* alignment depicted, 12-46, 9-70, 1-7 and 1-5 polymorphic sites, respectively that served as markers to distinguish between these genuine and adulterant species. Though *rbcL* had the least number of polymorphic sites, only it could be successfully amplified in all the 4 traded samples of the cooling beverage tested. *rbcL* revealed that traded samples of Kudidan, Ludougen and Xiangsizi as genuine material while Shepaole was substituted (116).

Traceability of *Lycium barbarum,* a nutritious food from other species like *Lycium chinense* and *Lycium ruthencium* was done using ITS2 barcode. BLAST1, NJ tree and nearest distance analysis using the ITS2 sequences could successfully discriminate between these three species (117).

The increasing demand for non-Camellia teas has paved way for its substitution with adulterants resulting in allergic reactions. BLASTN and phylogenetic analysis based on

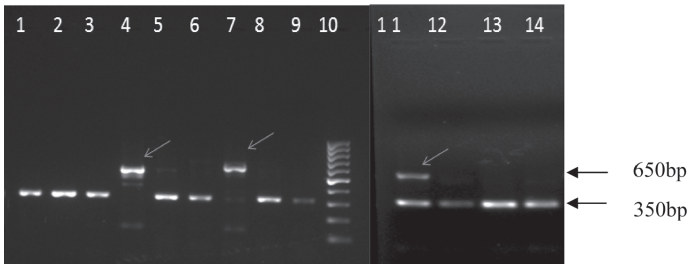

Fig. 2. Amplification of *psbA-trnH* locus (Lanes 1-3 -*Piper nigrum,* lane 4- *Capsicum annuum,* lane 5-Market sample1, lane 6- Market sample 2, lane 7- Market sample 3, lane 8- Market sample 4, lane 9- Market sample 5, lane 10 -100bp ladder, lane 11-Market sample 6, lane 12- Market sample 7, lane 13- Market sample 8, lane 14 -Market sample 9.

Table 1. Application of DNA barcoding in adulteration detection of medicinal plants

Application	Barcoding loci	Reference
Authentication of medicinal plants of Polygonaceae	*trnH-psbA*	(64)
Distinction between *Radix astragali* and its adulterants	ITS, *matK*	(65)
Authentication of *Taxillus chinensis*	ITS	(66)
Assessment of species admixture in *Phyllanthus amarus*	*trnH-psbA*	(67)
Authentication of *Ruta graveolens*	ITS	(68)
Differentiation of *Lonicera japonica* from its adulterant species	*trnH-psbA*	(69)
Molecular authentication of *Sabia parviflora* and its adulterants	*matK, rbcL, trnH-psbA*	(70)
Identification of *Gentianopsis paludosa* from its adulterants	ITS	(71)
Distinguishing medicinal plant *Paris polyphylla* from its Adulterant *Valeriana jatamansi*	*trnH-psbA*	(72)
Authentication of Black cohosh dietary supplements	*matK*	(73)
Authentication of commercialized medicinal plants in Southern Morocco	*rpoC1, matK*, *trnH-psbA*, ITS	(74)
Identification of *Solanum lyratum* from its substituent *Aristolochia mollissima*	ITS, *matK, rbcL, trnH-psbA, trnL-trnF*	(75)
Identification of *Dipsacus speroides* from three other species of genus *Dipsacus.*	ITS2	(76)
Authentication of *Sedum sarmentosum* from its adulterants	ITS2	(77)
Authentication of herbal materials	ITS2	(78)
Distinguishing *Boerhavia diffusa* from its adulterants	ITS	(79)
Differentiation of *Hemidesmus indicus* from its adulterant *Decalepsis hamiltonii*	ITS2	(80)
Authentication of natural health products	ITS, *rbcL*	(81)
Identification of tobacco seized from water pipes	*matK, rbcL*	(82)
Authentication of medicinal plants of Fabaceae	*trnH-psbA*	(83)
Discrimination of *Scutellaria baicalensis* from its adulterants	*trnH-psbA, rbcL*	(84)
Authentication of dietary supplement saw palmetto	*matK, rbcL*	(85)
Identification of processed medicinal materials in South Africa	*matK, rbcL*	(86)
Authentication of *Salvia divinorum* samples	*rbcL, trnL-trnF*	(87)
Detection of contamination & substitution in North American health products	*rbcL,* ITS	(59)
Differentiation of *Gentian a*s pecies traded as Guanlongdan from its adulterants	*rbcL, matK, trnL-trnF, trnH-psbA,* ITS	(88)
Discrimination of medicinal plant *Isatis indigotica* from its adulterants	ITS2, *rbcL, trnL-F*	(89)
Authentication of drugs used in traditional Chinese medicine	*rbcL*	(90)
Authentication of *Gingko biloba* dietary supplements	*matK*	(91)
Authentication of *Cassia* species used in traditional Indian medicine	*rbcL, trnH-psbA*	(92)
Molecular authentication of commercially sold medicinal Plants in Manila	*matK, trnH-psbA*	(93)
Authentication of medicinal materials sold in Dali fair	ITS2, *trnH-psbA*	(94)
Detection of adulteration in the drug trade of “Bala” drugproducts	*trnH-psbA*, ITS2	(95)
Authentication of *Swertia chirayita* and its adulterant species	ITS	(96)
Authentication of *Sida cordifolia* herbal products	*trnH-psbA*, ITS2	(97)
Authentication of commercial processed Glehniae Radix	ITS2	(98)
Authentication of medicinal plants used in herbal medicine in Brazil	*rbcL, matK,* ITS2	(99)
Assessment of adulteration in health products of *Cassia*, *Senna* and *Chamaecrista* in South India	ITS2	(100)
Discrimination between toxic aristolochiaceous and non-aristolochiaceous plant materials	ITS2, *trnH-psbA*	(101)
Survey of commercial Rhodiola products	ITS2, *trnH-psbA*	(102)
Molecular identification of Arisaematis Rhizoma and Pinelliae Tuber from its adulterants	*matK, rbcL*	(103)
Authentication of medicinal plant raw drugs used in Ayurvedic medicine	*rbcL*	(104)

Table 2. Application of DNA barcoding in adulteration detection of spices

Application	Barcoding loci	Reference
Identification and traceability of different spices like mint, sage, thyme, origanum and basil	*trnH-psbA, matK*	(60)
Authentication of *Ilicium verum* (star anise) from its adulterants	*trnH-psbA*	(106)
Traceability of commercial saffron	ITS	(107)
Detection of chilli adulteration in traded black pepper	*trnH-psbA, rbcL*	(105)
Discrimination of *Cinnamomum verum* from *C. cassia*	*rbcL*	(108)
Detection of adulterants in saffron	*trnH-psbA*	(109)
Tracing out adulterants in traded turmeric powder	ITS, *rbcL*	(110)
Detection of adulterants in traded nutmeg mace	*trnH-psbA*	(111)
Detection of adulteration in saffron	*matk, rbcL*	(112)
Authentication of saffron by mini-barcodes	*matK*, ITS1, ITS2	(113)

barcoding loci viz. *rbcL, matK, psbA-trnH* and ITS2 could identify non-Camellia teas from the samples screened thus providing a way to effectively label the commercially available samples and ensure safety to consumers (118).

DNA barcoding was used to solve a dispute in the international trade of roasted barley tea. Roasted barley tea consignment imported from China was rejected due to substitutions. Barcoding of the rejected barley tea samples using *rbcL, matK, psbA-trnH* and ITS2 loci revealed that out of the 13 batches of samples tested, 1 batch was substituted with *Morus* species. Out of the remaining 12 batches, 2 batches had only *Hordeum vulgare* while 10 had *H. vulgare* admixed with *Morus* spp*., Triticum* spp. *Avena sterilis* and *A. fatua* (119).

DNA barcoding technique was adopted to identify the source plants composition in processed honey using *rbcL* and *psbA-trnH* loci. BLAST analysis showed that the four honey samples studied are obtained from 39 plant species of genus such as *Castanea*, *Quercus*, *Fagus* and other herbal taxa. One out of the four samples studied also showed traces of genomic DNA from *Atropa belladonna,* a toxic plant thereby conforming the applicability of barcoding in certifying the food safety of commercial honey (120).

Authentication of seafood and meat : Efficiency of DNA barcoding in seafood traceability has resulted in its adoption as a method for authentication of fish based commercial products by the US Food and Drug Administration (121). Barcode based seafood authentication has been successful due to the availability of more than 70,000 reference sequences in the database, Fish Barcode of Life Initiative (FISH-BOl) (57). Many authors have reported mislabeling, substitution and adulteration of seafood, processed seafood and their byproducts like fish fillets using *cox1* gene based barcoding (122-126). Meat adulteration in US markets could also be traced using *cox1* based barcoding (63, 127) (Table3).

Conclusion

Globalisation has resulted in increased global trade accompanied by a rise in the unscrupulous practices of adulteration to attain economic gain resulting in eroding the perceived biological value and quality of the product besides corroding public faith. Food quality control is an essential prerequisite to safeguard the consumer's interests and health. DNA barcoding has been used an ideal tool to check food quality due to its simplicity, easiness, rapidness and sensitivity. It may soon emerge as a routine quality test for food authetication and traceability across the globe. The polymorphic sites found in the barcoding loci can be exploited to synthesize specific primers for developing diagnostic kits that may be handy for the food regulatory agencies in ensuring the quality of the traded food and agricultural commodities at the

Table 3. Applications of DNA barcoding in seafood and meat authentication

Application	Target gene	Reference
Identification of smoked fish products	*CO1*	(128)
Detection of market substitution in North American seafood	*CO1*	(129)
Identification of shark and ray fins using DNA barcoding	*CO1*	(130)
Detection of mislabeling in Amazonian commercial fish	*CO1*	(131)
Substitution of shark seafood products	*CO1*	(8)
Revelation of mislabeling in commercial fish products in Italy	*CO1, cytb*	(132)
Revelation of high rate of mislabeling in commercial fresh water catfish in Brazil	*CO1*	(133)
Revelation of high incidence of fish species misrepresentation and substitution in South African market	*CO1*	(134)
Market place substitution of Atlantic salmon in place of Pacific salmon in Washington	*CO1*	(135)
Revelation of market substitution of fish in Canada	*CO1*	(136)
Species authentication of catfish	*CO1*	(137)
Authentication of commercialized crab meat in Chile	*CO1*	(138)
Authentication of commercial seafood products	*CO1*	(139)
Species identification of some fish processing products in Iran	*CO1*	(140)
Detection of market substitution in salted cod fillets and battered cod chunks	*CO1*	(141)
Detection of mislabeled commercial fishery by-products in Philippines	*CO1*	(123)
Authentication of exploited grouper fish species	*CO1*	(142)
Authentication of processed and raw tuna from Indonesian markets	*CO1*	(143)
Revelation of mislabeling in Egyptian fish fillets	*CO1*	(144)
Detection of improper labeling and supersession of crab food served by restaurants in India	*CO1*	(145)
Authentication of snappers of West Atlantic	*CO1*	(146)
Revelation of commercial and health issues in ethnic seafood sold in the Italian market	*CO1*	(147)
Authentication of Porgies fish species of commercial interest on the international market	*CO1*	(148)
Governmental regulatory forensic program for identification of commercialized seafood in South Brazil	*CO1*	(124)
Detection of fish mislabelling and substitution in South Africa	*CO1*	(149)
Revelation of high substitution and mislabeling of croaker fillets in Brazil	*CO1*	(150)
Unmasking seafood mislabeling in US markets	*CO1*	(125)
Labelling accuracy in seafood retailers in Tasmania, Australia	*CO1*	(151)
Seafood identification using DNA barcoding revealed market substitution in Canadian seafood	*CO1*	(62)
Revelation of mislabeling of processed flat fish products in southern Italy markets	*CO1*	(152)
Authentication of heavily processed fish products using DNA mini barcoding system	*CO1*	(153)
Detection of mislabeled seafood products in Malaysia	*CO1*	(154)
Government commissioned authentication of fish products in Taiwan	*CO1*	(155)
Identification of species in ground meat products sold in US market	*CO1*	(63)
Mislabeling in Indian seafood	*CO1*	(156)
Revelation of mislabeling of game meat species in US markets	*CO1*	(157)
Revelation of fraud in commercial yak jerky sold in China using DNA barcoding	*CO1,*16SrRNA	(158)
Identification of common aquatic products and the supervision of its market trade in Central China	*CO1*	(159)
Revelation of mislabeling of imported fish productsin Nansha port of China	*CO1*	(160)
Revelation of mislabeling in seafood sold in Portuguese supermarket	*CO1*	(161)

port of origin/destination or in domestic whole sale or retail outlets.

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