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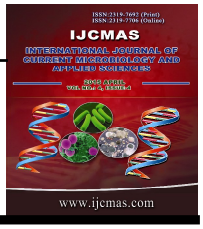

Review Article

A overview of Species Identification by DNA Barcoding

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A B S T R A C T

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The present mini-review is related with the recent work carried out in the field of DNA barcoding of different species. The barcoding of marine and freshwater fishes and honey bee samples are mostly studied. The study of DNA barcoding is essential to provide accurate toxinomy, biodiversity of species etc. Highlights of the study is Advances in DNA barcoding of fishes, honeybee. Snake etc are described and also Advantages and disadvantages of DNA barcoding is discussed.

Introduction

DNA barcoding is gaining more attention nowadays, because of its accuracy compared to other methods of taxonomy, though it has many limitations in the following paragraphs, work of earlier researchers has been described.

Three different minor groove binder (MGB) probe assays developed for rapid and accurate identification of the species commonly used for production of canned tuna, i.e. Yellowfin (Thunnus albacores), bluefin (Thunnus thynnus) and albacore (Thunnus alalunga) tunas. The assays targeting the mitochondrial cytochrome b gene were able to discriminate efficiently between the three species contained in fresh or canned tunas and did not react with other Scombroidei that were tested.

A correct species prediction was obtained even from artificial mixtures prepared with different amounts of the reference tuna species and subjected to the sterilization treatment. Testing of 27 commercial canned tunas by PCR-RFLP, MGB probe assays and sequence analysis showed a concordance of 100% between the last two techniques, whereas by using PCR-RFLP several samples were uncharacterized or mischaracterized (Terio V, et.al, 2010).

DNA barcoding aims to provide an efficient method for species-level identifications using an array of species specific molecular tags derived from the 5' region of the mitochondrial cytochrome c oxidase | (COI) gene. The efficiency of the method hinges on the degree of sequence divergence among

species and species-level identifications are relatively straightforward when the average genetic distance among individuals within a species does not exceed the average genetic distance between sister species. Fishes constitute a highly diverse group of vertebrates that exhibits deep phenotypic changes during development. In this context, the identification of fish species is challenging and DNA barcoding provide new perspective in ecology and systematic of fishes. (Hubert N., et.al, 2008)

Pesticides have an innate capacity to cause damage to biological system. The range of pesticide toxicity is large in aquatic organisms, among different fish species. The effect of sub lethal concentration of two different kinds of pesticides Fenvalerate (synthetic pyrethroid) and Monocrotophos (organophosphate) for 24, 48, 72 and 96 hrs were observed on DNA and RNA contents in selected tissues like gills, liver, kidney and muscle of freshwater fish Punctius arenatus (Day). Nucleic acids like DNA and RNA react differently with the various concentration and period of pesticide exposure. An overall decrease is nucleic acid was noted in these contents which are statistically significant. (Rathod ND., et.al. 2010)

Fisheries managers and scientists worldwide are struggling with a lack of basic information for many shark and ray species. One factor hampering the data collection is inaccurate identification of many chondrichthyan species and their body parts. Morphologically similar species, and specimens which are poorly preserved or have had key diagnostic features removed, can be difficult to identify. DNA barcoding as a method to identify shark species from dried fins, confiscated from vessel fishing illegally in Australian waters. 211 left pectoral fins were examined. 18 either did not provide a sequenceable product or yielded a microbial sequence, while 193 fins (91.5%) provided a chondrichthyan sequence. All of these could be matched to reference specimens in a DNA barcode database, and so were able to be identified. 27 species were detected, 20 species of sharks and seven species of rays. The most abundant species (22% of fins) was Carcharhinus dussumieri. Many of these species are listed on the World Conservation Union (IUCN) Red List and include one, Anoyxpristis cuspidata (3%), rated as critically endangered. Fishing authorities can use DNA barcoding to gather data on which chondrichthyan species are targeted by illegal fishers, information that will greatly assist in management and conservation. (Holmes BH., et.al. 2009)

With millions of species and their life-stage transformations, the animal kingdom provides a challenging target for taxonomy. Recent work has suggested that a DNA-based identification system, founded on the mitochondrial gene, (COI), can aid the resolution of this diversity. While past work has validated the ability of COI sequences to diagnose species in certain taxonomic groups. The results indicate that sequence divergences at COI regularly enable the discrimination of closely allied species in all animal phyla except the Cnidaria. This success in species diagnosis reflects both the high rates of sequence change at COI in most animal groups and constraints on intraspecific mitochondrial DNA divergence arising, at least in part, through selective sweeps mediated via interactions with the nuclear genome. (Herbert PDN., et.al. 2003)

Two hundred and seven species of fish, mostly Australian marine fish, were sequenced (barcoded) for a 655 bp region of the mitochondrial cytochrome oxidase subunit I gene (cox1.) Most species were

represented by multiple specimens, and 754 sequences were generated. The GC content of the 143 species of teleosts was higher than the 61 species of sharks and rays (47.1% versus 42.2%), largely due to a higher GC content of codon position 3 in the former (41.1% versus 29.9%). Rays had higher GC than sharks (44.7% versus 26.8%). Average within-species, genus, family, order and class Kimura two parameter (K2P) distances were 0.39%, 9.93%, 15.46%, 22.18% and 23.27%, respectively. All species had haplotypes characteristic of a congener. Although DNA barcoding aims to develop species identification systems, some phylogenetic signal was apparent in the data. In the neighbor-joining tree for all 754 sequences, four major clusters were apparent: chimaerids, rays, sharks and teleosts. Species within genera invariably clustered, and generally so did genera within families. Three taxonomic groups – dogfishes of the genus Squalus, flatheads of the family Platycephalidae, and tunas of the genus Thunnus – were examined more closely. The clades revealed after bootstrapping generally corresponded well with expectations. Individuals from operational taxonomic units designated as Squalus species B through F formed individual clades, supporting morphological evidence for each of these being separate species. The cox 1 sequencing, or 'barcoding', can be used to identify fish species. (Ward RD., et.al. 2005)

The sequences of 10 conservative regions (CR) of minicircles of 6 selected isolates of freshwater fish trypanosomes have typical organization of this region with high degree of sequence conservation. The comparison with CRs of other trypanosomatida showed that freshwater fish trypanosomes represent a compact separate group within the genus Trypanosoma. The alignment of all sequences obtained revealed, however, the existence of 2 types of CRs in sequenced minicircles, with the differences concentrated in a short region. (Kolesnikov AA., et.al., 1995)

The ancient DNA analysis to identify Pacific Salmon vertebrae to species in order to provide an important line of evidence that helps to establish the timing of seasonal residence at a Pacific Northwest Cost village site. Ancient DNA results from House 2 at Dionisio Point allow a characterization of the salmon fishery. Ten of eleven randomly selected smaller-sized salmon vertebrae were positively identified as sockeye salmon (Oncorhynchus nerka) while only a single pink salmon (Oncorhynchus gorbuscha) was identified. Of the 322 whole salmon vertebrae identified from House 2 occupation deposits during zooarcheological analysis, 58 percent measure less than 8.0 mm and 70 percent are less than 8.5 mm in maximum transverse diameter. Together with documented aspects of the material record from Dionisio Point, most notably the vertebrate fauna from House 2, the indications aspects of the material record from Dionisio Point, most notably the vertebrate fauna from House 2, the indication that sockeye was the primary focus of the Dionisio Point salmon fishery suggests the site was inhabited during the spring and summer. This approach to the identification of season-specific site occupation has the potential for application over much of the Northeast Pacific. (Ewonus PA., et.al. 2011)

Despite ongoing efforts to protect species and ecosystems in Cuba, habitat degradation, overuse and introduction of alien species have posed serious challenges to native freshwater fish species. In spite of the accumulated knowledge on the systematic of this freshwater ichthyofauna.

It is estimated that 40% of freshwater Cuban fish are endemic; however, this number may be ever higher. Partial sequences (652bp) of the mitochondrial gene COI were used to barcode 126 individuals, representing 27 taxonomically recognized species in 17 genera and 10 families. The mean conspecific, congeneric and confamiliar genetic distances were 0.6%, 9.1% and 20.2% respectively. Molecular species identification was in concordance with current taxonomical classification in 96.4% of cases, and based on the neighbor-joining trees, in all but one instance, members of a given genera clustered within the same clade. Within the genus Gambusia, genetic divergence analysis suggests that there may at least four cryptic species. In contrast, low genetic divergence and a lack of diagnostic sites suggest that Rivulus insulaepinorum may be conspecific with Rivulus cylindareus. Distance and character-based analysis were completely concordant, suggesting that they complement species identification. Overall, the results evidenced the usefulness of the DNA barcodes for cataloguing Cuban freshwater fish species and for identifying those groups that deserve further taxonomic attention. (Lara A., et.al. 2010)

The use of DNA in forensics has grown rapidly for human applications along with the concomitant development of bioinformatics and demographic databases to help fully realize the potential of this molecular information. Similar techniques are also used routinely in many wildlife cases, such as species identification in food products, poaching and the illegal trade of endangered species. The use of molecular techniques in forensic cases related to wildlife and the development of associated databases has, however, mainly focused on large mammals with the exception of a few high-profile species. There is a need to develop similar databases for aquatic species for fisheries enforcement, given the large number of exploited and endangered fish species, the intensity of exploitation, and challenges in identifying species and their derived products. A 500 bp fragment of the mitochondrial cytochrome b gene from representative individuals from 26 harvested fish taxa from Ontario, Canada, focusing on species that support major commercial and recreational fisheries sequenced. Ontario provides a unique model system for the development of a fish species database, as the province contains an evolutionarily diverse array of freshwater fish families representing more than one third of all freshwater fish in Canada. Inter and intraspecific sequence comparisons using phylogenetic analysis and a BLAST search algorithm provided rigorous statistical metrics for species identification. (Kyle CJ., et.al. 2007)

229 DNA sequences of (COI) from 158 marine fishes of Japan were employed to test the efficacy of species identification by DNA barcoding. The average genetic distance was 60-fold higher between species than within species, as (K2P) genetic distances averaged 17.6% among congeners and only 0.3% among conspecifics. There were no overlaps between intraspecific and interspecific K2P distances, and all sequences formed species units in the neighbor-joining dendrogram. Hybridization phenomena in two species (Kyphosus vaigiensis and Pterocaesio digramma) were also detected through searches in Barcode of Life Data Systems (BOLD). DNA barcoding provides a new way for fish identification. (Zhang JB., et.al. 2011)

The use of DNA barcodes for the identification of described species is one of the least controversial and most promising applications of barcoding. There is no

consensus, however, as to what constitutes an appropriate identification standard and most barcoding efforts simply attempt to pair a query sequence with reference sequences and deem identification successful if it falls within the bounds of some pre-established cutoffs using genetic distance. Since the Renaissance, however, most biological classification schemes have relied on the use of diagnostic characters to identify and place species. (Lowenstein JH., et.al. 2009)

Description and DNA barcoding of a new Sillago species, Sillago sinica (Perficormes: Sillaginidae), from coastal waters of China. Zoological Studies 50(2): 254-263. A new species of the genus Sillago, Chinese sillago, Sillago sinica sp. nov., was described using morphological methods and phylogenetic analysis of DNA barcoding of 53 specimens collected from the East China Sea (Wenzhou), Yellow Sea (Qingdao), and Bohai Sea (Dongying, China). Results of the morpholocial analysis (such as vertebrate counts, otoliths, etc.) showed that significant differentiation existed between S. sinica sp. nov. and 5 other Sillago spp. The mitochondrial DNA cytochrome oxidase subunit I (COI) gene was used as a DNA barcode to clarify the systematic of the genus Sillago. Results of the phylogenetic analysis showed that S. sinica sp. Nov. formed a monophyletic group as a distinct phylogenetic species. It was also suggested that the COI gene is an effective molecular marker for identifying Sillago species. (Gao TX., et.al. 2011)

Reliable recovery of the 5' region of the (COI) gene is critical for the ongoing efforts to gather DNA barcodes for all fish species. Test primer cocktails with a view towards increasing the efficiency of barcode recovery developed. Specifically, to evaluate the success of polymerase chain reaction amplification and the quality of resultant sequences using three primer cocktails on DNA extracts from representatives of 94 fish families. The results show that M13-tailed primer cocktails are more effective than conventional degenerate primers, allowing barcode work on taxonomically diverse samples to be carried out in a high-throughput fashion. (Ivanova NV., et.al. 2007)

Biogeographic controversies surrounding the wide-spread freshwater fish, Galaxias maculates, were addressed with DNA sequence data. Mitochondrial cytochrome b and 16S rRNA sequences were obtained from representatives of six populations of this species. Substantial levels of cytochrome b (maximum 14.6%) and 16S rRNA sequence divergence (maximum 6.0%) were detected between western Pacific (Tasmania-New Zealand) and South American (Chile-Falkland Islands) haplotypes. A considerable level of divergence was also detected between Tasmanian and New Zealand haplotypes (maximum 5.1%) and within and among Chilean and Falkland Island G. maculates (maximum 3.8%). The phylogenetic structure of haplotypes conflicts with the accepted pattern of continental fragmentation. Molecular clock calibrations suggest that haplotype divergences postdate the fragmentation of Gondwana. These findings point to marine dispersal rather than ancient vicariance as an explanation for the wide distribution. The phylogenetic structure of South American haplotypes was not consistent with their geographic distribution. Considering factors such as population divergence, population size, dispersal, secondary contact, and philopatry as potential causes of the high level of mtDNA nucleotide diversity in this species. (Waters JM., et.al., 1999)

RNA (rRNA) a new method for extraction microsporidian genomic DNA from infected host tissue is described for Nosema APIs. Complete DNA sequence data are presented for the small subunit gene (1242 bp), the internal transcribed spacer (33 bp), and the large subunit gene (2481 bp to a putative termination point). DNA sequence is also presented for the regions flanking the 5 end of the small subunit gene and the 3 end of the large subunit gene. The intergenic spacer is shown to be heterogeneous, showing variation in sequence and restriction sites rather than length and containing sequences repeats, which are a characteristic feature of intergenic spacers. The rRNA gene region of N. apis is shown to occur in a head-to-tail, tandemly repeated manner, as in other eukaryotes. This repeat unit is shown to be approximately 18 kb in length. (Gatehouse HS., et.al., 1998)

Honey is produced by honeybees from nectar and from secretions of living plants. It reflects the honeybee's diet and the local plant communities. Honey also shows different plant compositions in different geographical locations. A new method for studying the plant diversity and the geographical origin of honey using a DNA barcoding approach that combines universal primers and massive parallel pyrosequencing. To test this method, two commercial honeys, one from a regional origin and one composed of a worldwide mix of different honeys. The method proposed is fast, simple to implement, more robust than classical methods, and therefore suitable for analyzing plant diversity in honey. (Valentini A., et.al. 2010)

A mitochondrial DNA region encompassing part of the NADH dehydrogenase subunit 2 and isoleucine transfer RNA genes was PCR amplified, cloned, and sequenced for 14 morphometrically identified Apis mellifera subspecies and the New World "Africanized" honeybee. Twenty different haplotypes were detected and phylogenetic analyses supported the existence of 3 or 4 major subspecies groups similar to those based on morphometric measurements. However, some discrepancies are reported concerning the subspecies composition of each group. Based on the sequence divergence of Drosophila (2% per Myr) it was found that the four lineages may have diverged around 0.6 Myr. The variability found in this region shows that infer phylogenetic relationships and test hypotheses concerning subspecies origin, dispersion and biogeography. (Arias MC., et.al., 1996)

Two different genomic regions (ND2 mitochondrial gene and EF1-α intron) were PCR amplified, cloned and sequenced for the ten known honey bee species collected within their natural range distribution. DNA sequences were analyzed using parsimony, distane and maximum likelihood methods to investigate phylogenetic relationships with Apis. The phylogenetic analyses strongly supported the basic topology recoverable from morphometric analysis, grouping the honey bees into three major clusters: giant bees (A. dorsata, A. binghami, and A. laboriosa), dwarf bees (A. andreniformis and A. florae), and cavity-nesting bees (A. mellifera, A. cerana, A. koschevnikovi, A. nuluensis, and A. nigrocincta). However, the clade of Asian cavity-nesting bees included paraphyletic taxa. Exemplars of Apis cerana collected from divergent portions of its range were less related to each other than were sympatric A. cerana, A. nuluensis, and A. nigrocincta taxa. Nucleotide sequence divergence between allopatrically distributed western (A. mellifera) and eastern (A. cerana, A. koschevnikovi, A. nigrocincta, and A. nuluensis) cavity-nesting species, around 18% for the mitochondrial

gene and 10-15% for the nuclear intron, suggested an earlier divergence for these groups than previously estimated from morphometric and behavioral studies. This latter finding necessitates reevaluation of the hypothesized origin of extant European, African, and West Asian Apis mellifera. Sequence divergence between A. laboriosa and A. dorsata was consistent with behavioral data and supports the species status of A. laboriosa. (Arias MC., et.al., 2005)

The capability of PCR-TTGE to detect meat species in mixed animal samples was investigated as a necessary step in developing a method where the identification will be performed matching on the "DNA barcode" zone the sequences of resolved PCR products obtained from a limited set of "universal" primers. Exemplary mixtures from five important meat species were analyzed. At this stage more PCR reactions have to be applied on a sample but this should be easily improved using primers simultaneously (as a "cocktail") in a single reaction. (Colombo F., et.al., 2011)

Freshwater fauna of ancient lakes frequently contain endemic taxa thought to have originated during the long existence of these lakes, yet uncertainties remain as to whether they represent distinct genetic lineages with respect to more widespread relatives and to the relative roles of isolation and dispersal in their evolution. Phylogenetic analyses of sequence variation at nuclear and mitochondrial genes were used to examine these issues for the freshwater fish genus Barbus in two European ancient lake systems on the Balkan Peninsula. The nuclear and mitochondrial data yielded concordant phylogeographic patterns though incomplete sorting of nuclear haplotypes between some mitochondrial clades was detected. The distributions of two currently recognized species investigated here do not match the distributions of evolutionary lineages revealed by phylogenetic analyses. The Prespa barbell, Barbus prespensis, is not endemic ot the lakes Prespa as previously thought but is instead found to be widespread in the south eastern Adriatic Sea basin, with a distribution largely corresponding to the basin of the now extinct Lake Maliq historically connected with Lake Prespa. On the other hand, a cryptic phylogenetic subdivision in a widespread species, B. rebeli, was discovered to be more distant from B. rebeli than from other Barbus species and to be endemic to the system of connected lakes Ohrid and Shkodra. The division coincides with the hydrogeographical boundary delimiting distributions of other freshwater fishes, and we suggest that this newly discovered evolutionary lineage represents a distinct species. These findings support the emerging pattern that endemic taxa have evolved not through isolation of individual lakes, but in systems of currently and historically interconnected lakes and their wider basins. (Markova S., et.al., 2010)

Illegal trade of snake skin and uncontrolled hunting have instigated the extermination of many endangered snake species. Efforts to check illegal trade are often impeded due to lack of proper species identification methods. Hence, conservation strategies demand for authentic and quick identification techniques to trace the origin of the seized samples. The study employs DNA mini-barcoding as a method to identify some endangered snake species of India. A two sets of novel primers for targeting regions developed within the mitochondrial Cytochrome Oxidase I gene to produce 175 bp and 245 bp amplicons. 175 bp fragment was amplified in all 11 snake species studied while the 245 bp amplicon was obtained in

10 species. DNA mini-barcodes recovered from these amplicons enabled the identification of snake species by retrieving the sequences available in public databases. The similarity scores ranging from 98 to 100% (98% taken as threshold value for species identification) signify the consistency of these mini-barcodes in snake species identification. Moreover, the results of the validation study confirm the effectiveness of the technique in forensic perspective, where the diagnostic morphological features of the seized sample are often missing. (Dubey B., et.al., 2011)

In 2009, a disease outbreak caused by Aeromonas hydrophila occurred in 48 catfish farms in West Alabama, causing an estimated loss of more than 3 million pounds of food size channel catfish. Virulence studies have revealed that the 2009 isolates of A. hydrophila are at least 200-fold more virulent than a 1998 Alabama isolate AL98-C1B. However, up to now, no molecular markers have been identified to differentiate the highly virulent 2009 isolates from other isolates of A. hydrophila. To understand the genetic differences between the highly virulent 2009 isolates and the less virulent AL98-C1B at molecular level, PCR-select bacterial genome substractive hybridization was used in this study. A total of 96 clones were selected from the substractive genomic DNA library. Sequencing results revealed that the 96 clones represented 64 unique A. hydrophila sequences. Of the 64 sequences, three (hypothetical protein XAUC_13870, structural toxin protein RtxA, and putative methyltransferase) were confirmed to be present in the three virulent 2009 Alabama isolates but absent in the less virulent AL98-C1B. Using genomic DNAs from nine field isolates of A. hydrophila with different virulence as templates, two sequences (hypothetical protein XAUC_13870 and putative methyltransferase) were found to be only present in highly virulent A. hydrophila isolates, but absent in avirulent isolates. (Pridgeon JW., et.al., 2011)

A rapid and accurate method for the detection of Vibrio parahaemolyticus strains was developed, using multiplex PCR and DNA – DNA hybridization. Multiplex PCR was used to simultaneously amplify three diagnostic genes (tlh, tdh and fla) that serve as molecular markers of V. parahaemolyticus. Biotinyated PCR products were hybridized to primers immobilized on a microarray, and detected by chemiluminesce with avidin-conjugated alkaline phosphatase. With this method, forty-five samples were tested. Eight known virulent strains ($tlh^+/tdh^+/fla^+$) and four known avirulent strains ($tlh^+/tdh^-/fla^+$) of the V. parahaemolyticus were successfully detected, and no non-specific hybridization and cross-hybridization reaction were found from fifteen closely-related strains ($tlh^-/tdh^-/fla^+$) of the Vibrio spp. In addition, all the other eighteen strains of non-Vibrio bacteria ($tlh^-/tdh^-/fla^-$) gave negative results. The DNA microarray successfully distinguished V. parahaemolyticus from other Vibrio spp. (Wang R., et.al., 2011)

The ivory industry is the single most serious threat to global elephant populations. A highly sensitive species-specific real-time PCR assay has been developed to detect and quantify African elephant (Loxodonta Africana), Asian elephant (Elephas maximus) and Woolly Mammoth (Mammuthus primigenius) mitochondrial DNA from highly precessed samples involved in the international ivory trade. This assay is especially useful for highly processed samples where there are no distinguishing morphological features to identify the species of origin. Using species-specific Taqman® probes targeting a region

of the mitochondrial cytochrome b gene, An assay that can be used to positively identify samples containing elephant or Woolly mammoth DNA faster and more cost-effectively than traditional sequencing methods has been developed. Furthermore, this assay provides a diagnostic result based on probe hybridization that eliminates ambiguities associated with traditional DNA sequence protocols involving low template DNA. The real-time method is highly sensitive, producing accurate and reproducible results in samples with a few as 100 copies of template DNA. This protocol can be applied to the enforcement of the Convention of the International Trade of Endangered Species (CITES), when positive identification of species from illegally traded products is required by conservation officers in wildlife forensic cases. (Wozney KM., et.al., 2012)

Despite the potential model role of the green algal genus Codium for studies of marine speciation and evolution, there have been difficulties with species delimitation and a molecular phylogenetic framework was lacking. A 74 evolutionarily significant units (ESUs) are delimited using 227 rbcL exon 1 sequences obtained from specimens collected throughout the genus range. Several morpho-species were shown to be poorly defined, with some clearly in need of lumping and others containing pseudo-cryptic diversity. A phylogenetic hypothesis of 72 Codium ESUs is inferred from rbcL exon 1 and rps3-rpl16 sequence data using a conventional nucleotide substitution model (GTR + Γ + I), a codon position model and a covariotide (covarion) model, and the fit of a multitude of substitution models and alignment partitioning strategies to the sequences data is reported. Molecular clock tree rooting was carried out because out-group rooting was probably affected by phylogenetic bias. (Verbruggen H., et.al., 2007)

A putative serine protease gene was cloned from the genomic DNA of Vibrio parahaemolyticus FYZ28621.4. The gene consisted of 1779 base pairs and encoded a 592 amino acid protein. The gene was expressed in Escherichia coli. The expressed protease was purified by Ni-NTA His-Bind Resin column and showed a 63 kDa band on SDS-PAGE. The protease exhibited proteolytic activity on gelatin agar plate and showed maximal proteolytic activity at pH 8.0 and 37^0C. It hydrolyzed N-α-benzoyl-$_L$-tyrosine ethylester (BTEE) and N-acetyl-$_L$-tyrosine ethylester (ATEE). Mutants conserved residues Asp^{51} (Asp^{51}-Asn). His^{89} (His^{89}-Asp) and Ser^{318} (Ser^{318}-Leu, Ser^{318}-Pro) lost proteolytic activities completely. The protein was confirmed to belong to serine protease. The purified serine protease was toxic to zebrafish with a LD_{50} of 15.4 μg/fish. A DNA vaccine was constructed by inserting the mutated serine protease (Ser^{318}-Pro) gene into pEGFP-N1 plasmid. The pEGFP-N1/m-vps was transfected in HeLa cells. The serine protease was confirmed to be expressed by fluorescence microscopy observation and Western blotting analysis. The pEGFP-N1/m-vps was further observed to express in muscle of the injected turbot (Scophthalmus maximus) by Western blotting seven days after immunization. Efficient protection against lethal V. parahaemolyticus challenge was observed on the vaccinated turbot with pEGFP-N1/m-vps, with the highest relative percent survival (RPS) of 96.11%. Significant specific antibody responses were also observed in the turbot vaccinated with the DNA vaccine. The results indicated that the serine protease might ba a potential virulence factor and could be used as an efficient vaccine candidate for the disease

control caused by V. parahaemolyticus. (Liu R., et.al., 2011)

Zooplankton species diversity and distribution are important of environmental change in the Arctic Ocean, and may serve as 'rapid-responders' of climate-induced changes in this fragile ecosystem. The scarcity of taxonomists hampers detailed and up-to-date monitoring of these patterns for the rarer and more problematic species. DNA barcodes (short DNA sequences for species recognition and discovery) provide an alternative approach to accurate identification of known species, and can speed routine analysis of zooplankton samples. During 2004-2008, zooplankton samples were collected during cruises to the central Arctic Ocean and Chukchi Sea. A ~700 base-pair region of the mitochondrial cytochrome oxidase I (mtCOI) gene was amplified and sequenced for 82 identified specimens of 41 species, including cnidarians (six hydrozoans, one scyphozoan), arthropod crustaceans (five amphipods, 24 copepods, one decapods, and one euphausiid); two chaetognaths; and one nemertean. Phylogenetic analysis used the Neighbor-joining algorithm with (K-2-P) distances, with 1000-fold bootstrapping. K-2-P genetic distances between individuals of the same species ranged from 0.0 to 0.2; genetic distances between species ranged widely from 0.1 to 0.7. The mtCOI gene tree showed monophyly (at 100% bootstrap value) for each of the 26 species for which more than one individual was analyzed. Of seven genera for which more than one species was analyzed, four were shown to be monophyletic; three genera were not resolved. At higher taxonomic levels, only the crustacean order Copepoda was resolved, with bootstrap value of 83%. The mtCOI barcodes accurately discriminated and identified known species of 10 taxonomic groups of Arctic Ocean holozooplankton. A comprehensive DNA barcode database for the estimated 300 described species of Arctic holozooplankton will allow rapid assessment of species diversity and distribution in this climate-vulnerable ocean ecosystem. (Bucklin A., et.al., 2010)

Sea cucumbers are regarded as traditional delicacies, medicine and aphrodisiacs in Asia over many centuries. Generally, sea cucumbers are gutted, boiled and roasted, then preserved through drying, smoking or freezing. Thus, it is very difficult clearly the species of processed sea cucumber on the basis of their morphology. FINS methodology was developed as a tool to assess the incidence of incorrect labeling of sea cucumbers belonging to family Holothuriidae in commercial food products. The result showed that 7 samples were incorrectly labeled (63.6%). Moreover, the technique allows the genetic identification of more than 40 species from all over the world. (Wen J., et.al., 2011)

Various morphotypes of Contracaecum (Nematoda) larvae in different developmental stages described and then genetically characterized using sequence data obtained from the first and second internal transcribed spacers (ITS-1 and ITS-2) of nuclear ribosomal DNA (rDNA). The alignment of ITS-1 and ITS-2 sequence data for larvae investigated with those from well characterized adults in previous studies showed that fourth-stage Contracaecum larvae from the Australian pelican, Pelecanus conspicillatus, had the same sequence as Contracaecum microcephalum reported from the little pied cormorant, Phalacrocorax melanoleucos. In addition, four different morphotypes of third-stage larvae, including types I to IV, were found in various species of fish. Contracaecum type I larvae were composed to two distict

genotypes; those collected from mullet, Mugil cephalus, had the same sequence as C. multipapillatum, and those from an unknown species of hardyhead (family Atherinidae) were C. pyripapillatum. Contracaecum type II larvae from mackerel, Scomber australasicus, had the same sequence as adult C. ogmorhini sensu strict, found in the Australian and New Zealand fur seals (Arctocephalus spp). Contracaecum type III larvae from flathead, Phalacrocorax carbo. The approach used herein was aneffective means of matching incompletely identifiable larval nematodes with reference sequences and provides a basis for exploring the composition of populations of anisakid larvae in fish as well as their ecology, particularly their life cycles and transmission patterns. (Shamsi S., et.al., 2011)

Accurate delimitation of species is a critical first step in protecting biodiversity. Detection of distinct species is especially important for groups of organisms that inhabit sensitive environments subject to recent degradation, such as creeks, springs, and rivers in arid or semi-desert regions. The genus Dionda currently includes six recognized and described species of minnows that live in clear springs and spring-fed creeks of Texas, New Mexico (USA), and northern Mexico, but the boundaries, delimitation, and characterization of species in this genus have not been examined rigorously. The habitats of some of the species in this genus are rapidly deteriorating, and many local populations of Dionda have been extirpated. Considering the increasing concerns over degradation of their habitat, and pending a more detailed morphological revision of the genus, we undertook a molecular survey based on four DNA regions to examine variation over the range of the genus, test species boundaries, and infer phylogenetic relationships within Dionda. Based on analyses of two mitochondrial (cytb and D-loop) and two nuclear (Rag1 and S7) DNA regions from specimens collected throughout the range of Dionda, identified 12 district species in the genus. Formerly synonymized names are available for two of these species, and four other species remain undescribed. The limited distribution of several of the species, coupled with widespread habitat degradation, suggests that many of the species in this genus should be targets for conservation and recovery efforts. (Schonhuth S., et.al., 2012)

For comparative primatology proper recognition of basal taxa (i.e. species) is indispensable, and in this the choice of a suitable gene with high phylogenetic resolution is crucial. For the goals of species identification in animals, the cytochorme c oxidase subunit 1 (cox 1) has been introduced as standard marker. Making use of the difference in intra- and interspecific genetic variation – the DNA barcoding gap – cox1 can be used as a fast and accurate marker for the identification of animal species. For the Order Primates comparing the performance of cox1 (166 sequencies; 50 nominal species) in species-identification with that of two other mitochondrial markers, 16S ribosomal RNA (412 sequences, 92 species) and cytochrome b (con: 547 sequences, 72 species). A wide gap exist between intra- and interspecific divergences for both cox1 and cob genes whereas this gap is less apparent for 16S, indicating that rRNA genes are less suitable for species delimitation in DNA barcoding. For those species where multiple sequences are available there are significant differences in the intraspecific genetic distances between different mitochondrial markers, without, however, showing a consistent pattern. (Nijman V., et.al., 2010).

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