



DNA BARCODING FOR SEPIA OFFICINALIS ASSIGNMENT

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Worldwide, fisheries are one of the lucrative and remunerative sectors for millions of people. It provides employment and proteins for millions more. So, management of this sector is therefore a critical activity and the maintenance of resources is getting more priority today. Unfortunately, this sector is facing huge pressure from different sources, lead to instability in its components in both biotic and non-biotic. It was evident that over 60% of major marine fisheries have been browbeaten or over exploited (FAO 1997). The group known as cephalopods (class Cephalopoda) is the most complex in the phylum Mollusca, and indeed, in all of the invertebrate phyla. Cephalopods first appeared as a separate molluscan taxonomic entity, the nautiloids, in the Upper Cambrian period (over 500 million years ago), but more than half of these ancestors were already extinct by the end of the Silurian, 400 million years ago, when only the nautilus survived. Although there is a long fossil record of many different groups, all living cephalopods belong to two 'subclasses': the Coleoidea, which includes the major groups known as squids, cuttlefishes *sensu lato*, octopods and vampires, and the Nautiloidea, containing two genera, *Nautilus* and *Ilonautilus*, the only surviving cephalopods with an external shell. At the present time the status and understanding of the Systematics and Classification of the recent cephalopod is under considerable discussion. The families of living cephalopods are, for the most part, well resolved and relatively well accepted. Species-level taxa usually can be placed in well-defined families. The higher classification, however, still is not resolved. The classification above the family level is cotroversial and a broad consensus still needs to be achieved. This situation is not unexpected for a group of organisms that has undergone explosive research attention in recent decades. The *sepia officinalis* is a marine animal which usually dwells on shallow reefs or channels up to 80 feet deep. Some populations migrate from deep to shallow waters in the summer. They are often found on the bottom of the water buried in a thin layer of sand. Cuttlefish are soft bodied molluscs with short, flattened bodies and a large head. Cuttlefish skin is soft and delicate, and slides easily back and forth over an oval mantle of muscle that is attached to the rigid, internal cuttlefish bone. It is covered with chromatophores or pigment sacs which allow the cuttlefish to change color for camouflage purposes, mating rituals and to show emotions.

The *Sepia officinalis* is one of the important species of cephalopod living in the south peninsular coast of India. They are having high economic value. But the productions in terms of capture were declining in past few years not only those members of these group members were nearing extinct. It is mainly because of the variations among themselves and the sudden environmental changes. Now, this population number is declining in trend because of the environmental threats like pollution, temperature variation, over hunting, exploitation of breeding grounds and illegal fishing methods. So they are in urge to conserve from the above threats. So the present study is intended to conserve these populations through gene pools. In India, the *Sepia officinalis* population biology and structure is not studied and reported yet. Apart from that, lot of new conservation methods are developed worldwide but they are not properly used in India. For this reason, these *Sepia Officinalis* population is in these resources.



In many organisms, some genes in the mtDNA also seem to accumulate mutations more rapidly than do nuclear genes. In other words, it provides markers with greater variability and sensitivity to drift, and is therefore more likely to show differences between populations or species; this makes mitochondrial gene attractive for both systemic and population genetic studies. Actually, the inadequate state of fish systematics partly stems from the lack of comparative morphological studies. Many researchers have recently reported on fishes using mtDNA sequences to reconstruct intra and interspecific phylogenies and the mitochondrial COI gene or 16S rRNA region sequences have proven useful and adequate in resolving relationships among closely related taxa (Siti *et al.*, 2015). In this study, we examined phylogenetic relationships of fishes using 16S rRNA mitochondrial gene to elucidate species relationships and examine congruence of previous phylogenetic hypotheses.

Stop codons were absent from all amplified sequences of 16SrRNA and all the amplified sequences were nearly 540 bp in length. This suggests NUMTs (Nuclear DNA Sequences originating from mtDNA) were not sequenced. Because vertebrates NUMTs are typically smaller than 600bp (Richly and Leister, 2004). In addition, because of the higher copy number of mitochondrial 16S rRNA Sequences, prior barcode studies have shown that NUMTs are detected in a very small percentage of species. Moreover when detected, NUMTs regularly show indels or diagnostic mutations (eg:- stop codons) that reveal their presence. So, in the present study, we detected no signs of pseudogenes.

According to 16S rRNA, Analysis on the genetic distances between species showed a variation of 10.2% between the two unknown study materials (MVBIOSF1 and MVBIOSF2) and 29.3% and 30.3% variation between the study materials and *Micrixalus fuscus* (Outgroup). The genetic variation between study material “MVBIOSF1” and *Channa striata* was found to be 0.00 and that of MVBIOSF2 and *Etroplus suratensis* was also 0.00. These results suggest that study material “MVBIOSF1” could be *Channa striata* and MVBIOSF2 be *Etroplus suratensis*. These results are well within the barcode threshold value of 3.5% for allowed variation for a single species due to metapopulation variation occurs in different populations of the same species. Considering the high rate of discovery of new species of fishes and prevailing taxonomic ambiguities, DNA barcoding can be a useful tool to speed up the initial recognition of new units that may represent undescribed species. For this purpose, it is necessary to define threshold values that ideally provide a sharp distinction between intraspecific and interspecific divergence values. If an unknown sequence differs from the closest reference sequence by a divergence above the threshold, the individual from whom the sequences were obtained belongs to a candidate species, which means that its taxonomic status merits further investigation. Bradley & Baker (2001), for mammals, set this threshold at 11% for the cytochrome b gene, whereas Hebert et al. (2004) propose a COI threshold of only 2.7% for birds.

In 16S rRNA tree, poorly supported nodes must be considered only as an indication of what the relationships between major groups could be. Because weakly supported clade is not very robust and cannot be taken as strong evidence for the phylogenetic relationships it denotes. But we can propose a discussion of the possible implications of this cladogram, should its major features be confirmed in the future.



In addition, the mitochondrial genome is inherited as a single unit (Awise 2000). This means that processes that may mislead phylogenies based on a single gene may extend to every gene in the mitochondrial genome. For example, introgression (hybridization) can occur between species that are not each other's closest relatives, causing a gene from one species to appear in the genome of another and causing a phylogeny based on this gene to incorrectly show these distantly related species to be sister species. When mitochondria introgress, every single gene in the mitochondrial genome will tend to suggest this same misleading pattern. In summary, although mitochondrial data may work very well in many cases, to be certain that the relationships have been correctly inferred; it is invaluable to have other types of data as well.

Certainly, DNA barcoding is unable to provide a fully reliable species identification in fishes, especially if reference sequences do not cover the entire genetic variability and geographic distribution of a species. However, the same is true for any other morphological or morphometric identification method. Case studies are needed to estimate more precisely the margin of error of molecular identification of fishes. Partial sequence information of 16S rRNA gene can be used as a diagnostic molecular marker in identification and resolution of taxonomic ambiguity of fishes. The study has also supported the claim of robustness of universal primers for 16S rRNA mt genes.

Great deal of fundamental and applied researches on cephalopods, such as fauna, systematics, morphology, embryology, population genetics, and biodiversity have been completed since 1960s (Zheng *et al* 2001a, 2001b, Zheng *et al* 2004). But these study is still lacking in Indian Peninsular coast cephalopods, especially sepia populations

Fig. 1 Agarose gel pictures of Genomic DNA (a, b, c = Specimen number)

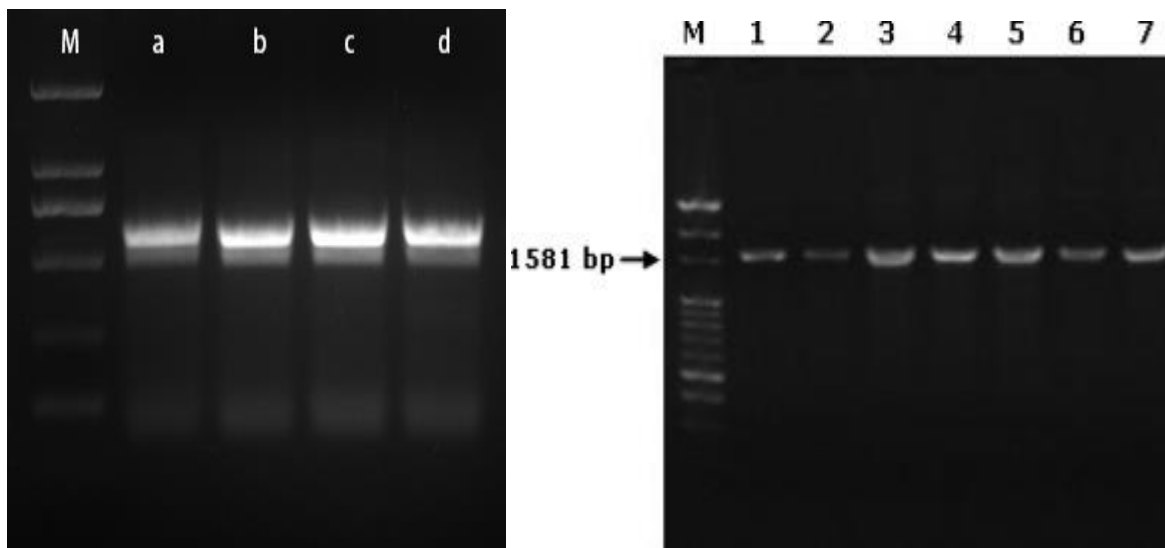


Fig. 2 Gel picture of 16S rRNA gene amplified products (1, 2,3 = samples;)



Summary and conclusion

DNA bar coding is a new approach of taxonomic identification which have provided a wealth of information about the organisms around us. With the help of this new approach we can identify the undiscovered and extinct species and conserve them. Mitochondrial DNA are used for identification because of its maternally inheritance property. 16SrRNA gene can be used as universal molecular marker for identification of any organism around us. Thus, in future DNA bar coding will be an easier approach in identifying the species and even a non-taxonomist can be expertise in identifying and conserving the extinct ones and thus increase our ecological diversity.

These ideas highlight an important aspect of the genetic conservation of this species. The practice of releasing cultured fish into the wild is widespread, but without a careful genetic analysis, it may damage the goals of preservation, leading to the homogenization of populations and decreasing species diversity (Taggart and Ferguson, (1986). Furthermore, it is important to recognize that different scenarios require different measures. It is one of the report indicating that the variation of *S.officinalis* populations and there lack of references in the same. Hence, the aim of conservation programs should be to develop an integrated strategy that conserves as much genetic diversity within the species as possible, and ensures the presence of utilizable fish resources. (Hansen and Loeschcke,(1994). The results promise to be useful for the fishery management, aquaculture and stock conservation of this species. The useful information obtained in this study will offer insights into how to fine-tune conservation and fishery management measures for this species and resource in the future

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