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Research Article

STUDIES ON PROXIMAL COMPOSITION AND DNA BARCODING OF MARINE SHRIMPS FROM THONDI, TAMILNADU, INDIA

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ABSTRACT

The shrimp is one of the most popular species as it is almost a part of every nation's traditional meal, rich in protein and minerals. Because of their many nutritional benefits, prawns are considered to be the healthiest foods in the world. They also have great economic value as they earn valuable foreign exchange. Four different types of marine shrimps were identified morphologically and molecularly discriminated by mitochondrial COI (cytochrome C oxidase subunit) gene. DNA barcoding is useful tool for identification and potential discovery of new species. The investigation principally entails with the proximate analysis such as moisture, ash, protein, carbohydrate, lipid and fatty acid content of four different types of marine shrimps (Type I - Grooved tiger shrimp, Type II - Mangrove or snapping shrimp, Type III - Glass shrimp and Type IV - Squilla mantis shrimp). The results showed that the high amount of moisture (72%), Protein (190.19 µg/g) and Lipid (167 µg/g) content were found to be in Type III marine shrimps compared to other three samples. Of these four sample shrimps, Ash (6.71 %), Carbohydrate (184 µg/g) and fatty acid (154 µg/g) content were found to be higher in Type IV sample. The study reconfirms the usefulness of DNA barcoding for the identification of marine shrimps. BOLD system analysis accurately identifies the species name and their similarities were checked with BLAST-NCBI. Each and every species showed 80% identity.

Keywords: Tiger shrimp, Glass shrimp, Marine shrimps, DNA barcoding, Snapping shrimp, Mitochondrial COI, Squilla mantis shrimp.

INTRODUCTION

Shrimp is one of the most popular species as it is a part of almost every nation's traditional meal rich in protein and minerals[1]. Generally, more than 10 million tons of crustaceans are produced annually for human consumption. Prawns, like most other crustaceans are able to change colour depending upon growth, background coloration and time of day due to chromatophores[2]. Grooved tiger prawns are subtropical species with pale brown to green brown body and having discrete white stripes along the top of the head, body and tail. The snapping shrimp may also be called symbiosis shrimp and pistol shrimp. Snapping shrimp are not easily sexed, but the males of many species are thought to have a larger pincher. The mantis shrimp or stomatopodis, a type of marine crustacean of the order Stomatopoda. Most species can grow to around 10 centimeters (3.9 in) in length, though a few species reach up to 38 cm. The largest ever caught has a length of 46 cm (18 in) in the Indian River near Fort Pierce, Florida of USA. The glass prawns are not readily available everywhere. Life span of false or glass prawn is 1 to 2 years. Glass shrimp size varies by age, but generally they grow to be about 1 ½ to 2 inches in length.

Biochemical assays and nutrients play a vital role in physical growth, development, maintenance of normal body function of physical activity and health[3]. Because of their many nutritional benefits[4], prawns are considered by a variety of health experts to be among the healthiest foods in the world. In contrast, the proportions of moisture and ash contents were higher in male prawns when compared with females. The level of DNA was found to be unchanged in both male and female prawns[5]. As far as prawns are concerned, the DNA barcoding is a useful tool in species identification and plays an imperative role for assessing non-described and cryptic species[6]. Barcoding of indicator species can be fruitful in the monitoring and abatement of marine pollution including coastal pollution. One main aim of DNA barcoding initiative is the discovery of new species[7].

This investigation principally entails with the proximate analysis, such as lipids, protein, fat, moisture, vitamins, minerals and carbohydrate content of the whole prawn, exoskeleton and edible portion of shrimp[8]. On the other hand, morphological characters may often be undergoing convergent evolution as they are under similar selective pressure. The freshwater shrimp genus Caridina is a challenge to taxonomists of all hues because of the confusion between intra and inter specific variation of characters. However, new techniques have been largely unable to prevent a much lamented crisis of taxonomy, which is an endangered species[9]. The study also reconfirms the usefulness of DNA barcoding for the identification of marine shrimps. BOLD system analysis accurately identifies the species name and their similarities were checked with BLAST-NCBI.

MATERIALS AND METHODS

Sample Preparation: A total of four different types of fresh marine shrimps were collected from the site of Thondi seashore situated in Rameshwaram coastal region of Tamil Nadu and the

samples were stored in 70% ethanol. The excess sample was placed at 4º C in refrigerator for further studies.

Analysis of Proximate composition of Shrimp Tissue: The proximate analysis are good indicators of physiological condition of an organism. The collected tissue sample was washed with sterile distilled water and then washed tissue sample was used for analysis of proximate composition such as moisture content, Ash content, crude protein[10], carbohydrate[11], lipid content (sulphophosphovani- Uin method) and fatty acid[12] as per adapted standard procedure.

SDS-Polyacrylamide Gel: This protocol describes the separation of proteins by SDS- polyacrylamide gel electrophoresis[13]. SDS is used with a reducing reagent and heat to dissociate the proteins. SDS-Polypeptide complexes from and migrate through the gels according to the size of the polypeptide. By using markers of known molecular weight, the molecular weight of the polypeptide chains can be estimated.

Genetic Analysis: The Genetic analysis was started with the isolation of DNA from the tissues using Phenol: Chloroform method. PCR analysis was carried out as per Kary mullis protocol[14]. PCR amplification of 16S rRNA of the obtained strain was performed using the primers LCOI1490F (5' – GGT CAA CAA ATC ATA AAG ATA TTG– 3') and HCOI2198R (5' – TAA ACT TCA GGG TGA CCA AAA AAT CA – 3'). PCR products were detected by 0.8% agarose gel electrophoresis and were viewed under UV transilluminator Primers

Gel Elution and Sequencing Analysis: MACHEREY- NAGEL PCR clean up kit was used to elute the PCR products from agarose gel. Eluted DNA of tissue sample was used for sequencing analysis by sequencing technology method [Sanger Method (Dideoxynucleotide chain termination)]. Sanger sequencing is a method generally used for sequencing DNA, where, target DNA is annealed to an oligonucleotide primer after denaturation and then extended by DNA polymerase using a mixture of normal dNTPs (deoxynucleotide triphosphates) and chain-terminating ddNTPs (dideoxynucleotide triphosphates). The synthesized DNA chains will varies in length and depends on random incorporation of dNTP.

Table 1: Proximate composition analysis of Shrimp samples

S. no	Sample Marking	Moisture content %	Ash content %	Protein concentration (µg/g)	Carbohydrate concentration (µg/g)	Lipid concentration (µg/g)	Fatty Acids (µg/g)
1	Type I	72	5.25	144.87	162	128	118
2	Type II	67	3.56	137.99	156	112	106
3	Type III	**83**	4.76	**190.19**	127	**167**	131
4	Type IV	53	**6.71**	103.15	**184**	98	**154**

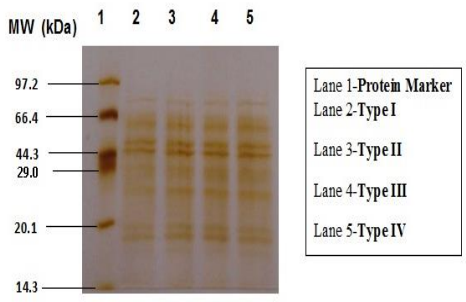

Figure 1: SDS PAGE depicting protein profile of Type I, Type II, Type III and Type IV casted with 10 % Agarose Gel Electrophoresis

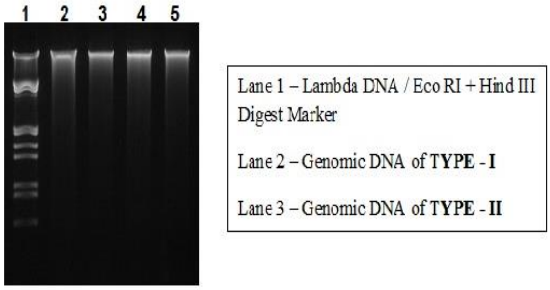

Figure 2: Genomic DNA showing Type I, Type II, Type III and Type IV casted in 1% Agarose Gel Electrophoresis

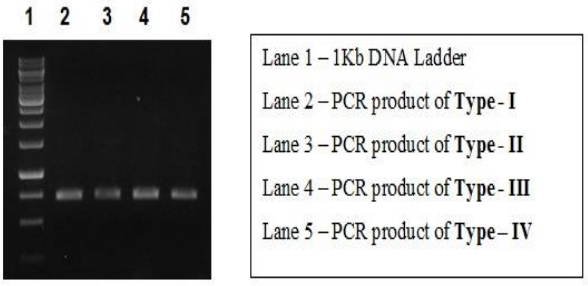

Figure 3: Amplified PCR of Type I, Type II, Type III and Type IV casted in1 % Agarose Gel Electrophoresis

RESULTS AND DISCUSSION

Proximate composition Analysis of Shrimp samples: The Proximate composition of processed tissue samples were estimated and the results are shown in Table 1. The results showed that the high amount of moisture (72%), Protein (190.19 µg/g) and Lipid (167 µg/g) content were found to be in Type III marine shrimps compared to other three samples. Of these four sample shrimps, Ash (6.71 %), Carbohydrate (184 µg/g) and fatty acid (154 µg/g) content were found to be higher in Type IV sample.

SDS-PAGE: Figure 1 represents the SDS-PAGE gel depicting the protein profiling of Type – I, II, III and IV samples

Isolation of Genomic DNA: Shrimp Genomic DNA was isolated by adopted manual method. Genomic DNA size is approximately 23130bp in length. The isolated genomic DNA size confirmed with Lambda DNA / Eco RI + Hind III Digest Marker by using agarose gel electrophoresis method. The isolated genomic DNA was used for PCR amplification technique. The isolated genomic DNA gel picture is shown in figure 2.

Polymerase Chain Reaction (PCR): The isolated shrimp genomic DNA was used for PCR amplification process. In this PCR Amplification technique used COI primers for synthesis of COI Gene product. This COI gene product size is approximately 750bp in length. The amplified PCR product size confirmed with 1kb DNA ladder by using Agarose gel electrophoresis method. Confirmed PCR product was used for elution process. Figure 3 shows the gel picture of amplified PCR product.

Gel Elution and Sequencing Analysis: PCR product was purified by MN kit method. The Eluted DNA was used for sequencing analysis. Eluted DNA was used for sequencing analysis. This Sequencing analysis was done by sanger method.

Type- I (GROVED TIGER SHRIMP): Based on NCBI BLAST Report COI Gene sequencing, Type I is *Peuaeus spp*. This species accepted name was identified and proved in world register of marine species (WRMS). Based on BOLD SYSTEM, this species is newly to the database. Blast nucleotide alignment displayed high similarities nearly 99% and bit score 1181.

Assembled sequence of COI gene –Type I

GACCAAAAAATCAAAATAAGTGTTGATATAGTACAGG
GTCTCCTCCACCGGCAGGGTCGAAGAAGGATGTATTT
AGATTTCGGTCTGTTAGAAGCATTGTAATAGCTCCTGC
TAGCACTGGTAAAGATAGAAGTAGAAGCAGGGCAGTA
ATAAATACTGCTCAAACGAACAGAGGTATTCGGTCTA
TAGTTATTCCAGTAGATCGTATATTAATAACGGTTGTT
ATAAAATTTACGGCTCCTAAAATAGATGATACACCTGC
TAGATGAAGTGAGAAGATCCCTAAGTCTACTGAAGCA
CCTGCGTGAGCAATTCTGGCAGATAAAGGAGGGTATA
CTGTTCAACCTGTTCCTACTCCTCTTTCTACTATACCTC
TAGATAAAAGTAAGGTTAGTGAAGGAGGTAAAAGCCA
GAAGCTTATATTATTTATACGAGGGAAAGCTATATCTG
GAGCTCCTAATATTAGAGGAACTAGTCAGTTACCAAA
TCCTCCAATCATGATAGGTATAACTATGAAGAAAATTA
TAACAAAAGCGTGAGCTGTTACAACCACATTATAAAT
TTGATCATCTCCAATAAGTCTACCAGGTTGACCTAATT
CAGCACGAATAATAAGTCTAAGAGCTGTACCTACTATT
CCAGCTCAAGCACCGAAAATGAAATATAATGTTCCAA
TATCTTTATGATTTGTTGAC

Type- II (MANGROVE OR SNAPPING SHRIMP): Based on NCBI BLAST Report COI Gene sequencing, type II is *Alpheus spp*. This species accepted name was identified and proved in world register of marine species (WRMS). Based on BOLD SYSTEM, this species is newly to the database. Blast nucleotide alignment displayed high similarities nearly 84% and bit score 634.

Assembled sequence of COI gene –Type II

TTGGATGTTTTGGTTTAGATGGGATCACCGCCGCCGGC
AGGGTCGAAAAATGCTGTATTTAGGTTTCGGTCTGTTA
AGAGTATAGTGATTGCTCCGGCTAGAACTGGTAGCCTT
AAGAGGAGTAGAATTGCTGTTAGGAATACAGCTCAGA
CAAATAGGGGCATTCGGTCTATAGTTATACCGGTAGAT
CGTATGTTAATAACTGTTGTTATGAAGTTAACTGCTCC
TAAAATTGAGGAAACACCTGCTAAGTGAAGTGAGAAA
ATACCCAGGTCTACTGAGGCTCCGGTATGGGCGATGC
CAGCTGATAGGGGTGGGTATACGGTTCATCCTGTGCCC
ACTCCTCTTTCTACTAGGCCTCTTGAGAGGAGAAGTGT
CAGGGATGGAGGGAGGAGTCAAAATCTTATATTGTTG
ATACGGGGGAAGGCCATGTCTGGGGCTCCTAGCATCA
GGGGAACTAATCAGTTTCCAAAGCCTCCTATTATGATT
GGTATAACTACGATAAAGTTTCTTACTTTTGCGTGGTA
CTCTTATCAGCTCCTTATTCGTTTTTTGTTCTTTGCCATT
TATTCCTTCCTGTCTTGCCCGATTTCACTCCTAAATTTT
TATCATATACTTTGCTTTCCACAAATTTTGGCCTCATTT
ACCAAATTAATATGTAATTATGCTTATATATATTTTAT
AATTTGTGAAACATTTACAACCTCTGAAGCTTTAATTT
TCTAATGTCTATTTTTTATTTTACTTCTTTTTGTGAATAT
TTTAAAACCACCAT

Type- III (SQUILLA MANTIS SHRIMP): Based on NCBI BLAST Report COI Gene sequencing, Type III is *Miyakella spp*. This species accepted name was identified and proved in world register of marine species (WRMS). Based on BOLD SYSTEM, this species is newly to the database. Blast nucleotide alignment displayed high similarities nearly 98% and bit score 1171.

Assembled sequence of COI gene –Type III

ACAAATCATAAAGATATTGGAACTTTATATTTTATTTT
AGGAGCTTGATCAGAATAGTAGGTACAGCCCTTAGTTT
AATCATTCGAGCAGAACTAGGACAACCAGGTAGTTTA
ATTGGAGACGACCAAATTTATAATGTTATCGTTACAGC
CCTGCTTTCATTATGATTTTTTTTATGGAATACCAATTA
TAATTGGAGGGTTCGATGACTAGTCCCTCTTATACTAG
GAGCTCCTGATATAGCTTTCCCTCGAATACAATATGAG
ATTCTGATTATTACCACCTGCTCTCACGCTTTTACTCTC
AAGTGGCTTAGTAGAAAGAGGAGTAGGAACAGGATG
AACGGTTTACCCTCCTTTATCTGCAGGAATTGCACATG
CAGGGGCGTCCGTGGATATGGGTATTTTTTCTTTACAT
CTAGCAGGGGCCTCTTCGATTTTAGGGGCAGTAAACTT
TATTACTACCGTGATCAATATACGATCTAATGGAATAA
CTATGGACCGTATACCCTTATTCGTATGAGCTGTTTTT
ATTACAGCTATTTTATTATTACTCTCTCTGCCTTTTAGC
AGGCCATCACTATGTTATTACCGAAACTTAAACACTCT
TTCTTCGATCCTGCTGGAGGAGGAGACCCTGTTTTATA
CCAACACTTATTTTGATTTTTTGGTCACCTGAAAGTTA

Type- IV (FALSE WHITE OR GLASS SHRIMP): Based on NCBI BLAST Report COI Gene sequencing, Type IV is *Artemesia spp*. This species accepted name was identified and proved in world register of marine species (WRMS). Based on BOLD SYSTEM, this species is newly to the database. Blast nucleotide alignment displayed high similarities nearly 86% and bit score 289.

Assembled sequence of COI gene –Type IV

CCGATTCTTTGTATTTGGCGCTTGGGCCGGATGGTGGG
GACAGCGCTCGATTACTAATCCGAGCCGAACTCGCGT
AAATGTAGGCTTATTGGCAACGCAAATCTATAATGTTA
TCGTAACAGCCCACGCCTTTGTAATAATTTTCTTCATA
GTTATCCAATCATAATTGGAGGCTTTGGAAACTGATTA
GTTCCCCTGATGCTAGGAGCCCCAGACATGGCCTTCCC
CCGTATAAACAATATAAATTTTGACTCCTCCCTCCATC
CCTGACACTTCTCCTCTCAAGAGGCCTAGTAGAAAGA
GGAGTGGGCACAGGATGAACCGTATACCCACCCCTAT
CAGCTGGCATCGCCCATACCGGAGCCTCAGTAGACCT
GGGTATTTTCTCTCTTACCTGGGCAGGTGTTTCGCTAA
TTTGGGTAGCAGATAGATCTGCACATCAGGTAGTGGT
GCTGATGTATCGGACTGTTCTTAGTTCGGATTGTAGCT
GCGACTCGCCTACATGAATCTGGTATGGGTAGTAATCG
TGAATCAGATGCCTCGGTGAATACGTTCCCGGGCCTTG
TACACACCGCCGCGTCACA

CONCLUSION

The results of this study shown that conventional DNA barcoding is an efficient tool that can be used to identify food components and validate label information contents. Our study clearly shown an efficiency universal barcode region (COI). In general mitochondrial DNA barcoding is simple, robust and cost effective, which makes it suitable for sea foods authentication assay, even with degraded samples, processed products or small portions of any species. For species identification by means of DNA based assays, there are two basis approaches: sequencing of multiple barcodes or detection of unique markers. The current study suggested that moisture, protein, lipid content was found to be higher in Type III and ash, carbohydrate, fatty acid content was found to be higher in type IV. As per nutritional status was concerned, this was commercially sound species and also has great export value.

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