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PHYLOGENETIC ANALYSIS OF TWO *SPECIES* OF SEA HORSE GENUS: *HIPPOCAMPUS* USING CYTOCHROME B OF MITOCHONDRIAL DNA

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ABSTRACT

Phylogenetic analysis of two species sehorse, genus Hippocampus analysed in the present work using cytochrome b of mitochondrial DNA. The partial sequence which is having 696 were sequenced for two species of genus hippocampus namely *Hippocampus kuda* and *Hippocampus kuda*. The phylogenetic relationship of the genuss hippocampus with special emphasize to Indian coastal water and to explore the genetic connectivity. From the phylogenetic tree *Hippocampus kuda* were found to be closely related to the haplotype IND 338.5 where as *Hippocampus kuda* population is not unique distribution in nature from the phylogenetic tree it was observed that the mixed population of TH16 and JAP4 haplotype as indicated by their bootstrap values. The phylogenetic analysis revealed that among two species of *Hippocampus kuda* is unique distribution in Indian water and incase of *Hippocampus kuda* the population is mixed one.

KEYWORDS: *Hippocampus kuda, hippocampus timaculatus, phylogenetic analysis, haplotype.*

1. INTERDUCTION

Seahorse belongs to group syngnathidae a teleost family, it has a number of synapomorphic morphological characters distinguishing from most of other syngnathides these charecters include a prehensile tail, the absence caudal fin, position of the head at right angle to the trunk , a broad pouch sealed along with the med line and raised dorsal fin base. Seahorses are example os of marine species whose unusual biology and global distribution provide an

interesting frame work for the study evolutionary hypothesis pertaining to marine environment.[1] Sea horse species have longitudinal and latitudinal ranges but are considered feeble swimmers. Southeast area being a highly diverse area a Wallace line separates terrestrial flora and founa in to Asian and Australasian elements.[2] In contrast the organism are expected to show a northeast to southwest division perpendicular to the wallaces line reflecting leistocene separation of Indian oceans versus pacific oceans population.[3] A high marine diversity in south east Asia is found where at least 10 species of seahorse can be found. The high diversity may be due to complex geological history in some parts and pleiostocene sea level changes in others. Differences in phylogeographical patterns among the species may also occur. Based on specific habitats they occupy some may be shallow water species generally found in sea grass or mangroves or muddy less than 10 m deep and some may be found at least at the depth of 10 to 15m, we focused on two species *Hippocampus kuda* and *Hippocampus kuda* that are assumed to different in their dispersal capabilities based on contrasting habitats to know the phylo geographical variation of sea horse among the Indian water with other oceans for the most sea horses dearth of available ecological information.[4] The goal of the study is to use the molecular phylogeny to investigate the patterns of dispersal and variance. Molecular markers have contributed towards understanding the evolutionary process in marine realm. The cytochrome b gene has been used in large number of studies and then uses this molecular phylogenic study to provide credibility for the seahorse species designation and also find genetic connectivity

2. MATERIALS AND METHOD

2.1. Sample Collection

The sea horse sample were procured from fishers and traders from tuticorin, gulf of mannar, south east cost of india and a small piece of tail portion were stored in 70% ethanol. Tail is the part of seahorse from which DNA will be isolated. Tail part is taken for the reason that it can be regenerated in live seahorse hence facilitating the conservation of the organism.

2.2. Isolation of DNA

DNA isolation was carried out by taking 500 mgms of the sample was taken and chopped finely using a sterile blade and put in an 1.5ml tube the standard EDTA method was use for isolation of DNA. After isolation the quantification was carried out find concentration using spectrophotometer method. To check the quantity of the agarose gel electrophoresis was carried out with standard markers and quantified DNA was taken for the further amplification.[5]

2.3. Amplification of DNA by PCR

To amplify the DNA first primers was designed considering the some of the parameters: the GC content should be around 60%, annealing temperature is usually 5 degree centigrade below the calculated average temperature however it may vary in individual reactions. Inner self complementary hairpins of above and 4 and of dimer 8 should be avoided, primers terminus design is critical to PCR success as the primer extended from the 3' end. The 3' end should not be complementary over greater than 3-4 base to any region of the primer used the reaction and must provide correct base matching to the template to amplify DNA we have used forward primers: CytbF: CTACCTGCACCATCAAATATTTC and reverse primers CytbR: CGGAAGGTGAGTCCTCGTTG. The PCR product was pre treatment because contains un amplified nucleotides by using Exo/Sap purification method.[6]

2.4. DNA sequencing

The purified product is sent for sequencing, the purpose of sequencing is determining the order of nucleotide in a particular gene. The sequencing reaction has three steps: denaturation, annealing and extension with the suitable oligonucleotides.

3. RESULTS AND DISCUSSION

3.1. Sample Collection

The sea horse sample was collected for the fishers and cleaned with tap water and fallowed by distilled water to remove surface infectants. The tail of the hippocampus is cut and finely chopped and stored in 70% alcohol.

3.2. Isolation of DNA

The isolation of DNA was carried out using EDTA method, the good quality of DNA was obtained and it was further quantified by using spectrophtometer method at 260 to 280nm absorbance.[7] The repeated isolation of DNA was done till obtaining good quantity of the DNA. After obtaining the good quantity of the DNA further subjected agarose gel electrophoresis with standard markers.[8]

3.3. Amplification of DNA

Genomic DNA was isolated from sea horse tissue using standard protocol with little modification. To amplify mitochondrial DNA cytochrome b (696 bases) partial gene the primers were synthesized by ABI 392 oligosynthesizer[9] and the best primers was obtained

are CytbF: CTACCTGCACCATCAAATATTTC and CytbR: CGGAAGGTGAGTCCTCGTTG.

3.4. Sequencing of the Amplicon

All amplicons was directly sequenced with both forward and reverse primers in separate reaction using bigDye terminator ready reaction cycle sequencing kit. Extended products were purified by alcohol precipitation fallowed by washing with 70% alcohol. Purified samples were dissolved in 10microlitre of 50% Hi-Di formamide and analyzed in automated DNA analyzers Sequence obtained were assembled using auto assembler to obtain the consensus sequence and these were translated in to amino acid sequences using SeqEd software.[10]

3.5. Nucleotide Substitution and genetic distance using phylogenetic analysis

The pair wise genetic distances calculated from cyt b sequences between *Hippocampus kuda* species and *Hipocampus trimaculatus.*[12] The overall percentage of synonymous and non synonymous substitutions rate was 0.53 the substitution rates at third codon position of Cytb gene was 0.02-3% the overall average of codons usage among the hippocampus species was 224. The results of phylogenetic analysis show that Indian population of *Hippocampus kuda* is not unique distribution it was observed from the phylogenetic tree.

4. CONCLUSION

The main objective of this work is to infer the genetic study and phylogenetic relationship of the genus Hippocampus with special emphasize to Indian coastal water using cytochrome b mitochondrial DNA and to explore genetic connectivity.[13] From, the phylogenetics of *Hippocampus kuda* were found to be closely related to the haplotype IND 338.5 where as *Hippocampus kuda* population is not unique distributed in nature from the analysis it was observed that the mixed population of Th16 and JAP4 haplotype as indicated by their bootstrap values.

7. REFERENCES

1. Arnason, E, P. Alsson S., Mitochondrial cytochrome b DNA sequence variation of atlantic cod gadus morhua from Norway Journal Mol. Ecol, 1996; 5: 715-724.
2. Avise J C the history and information of species Harvard University press Cambridge MA, 2000.

3. Baldwin J D Bass, A L Bowen BW Clark Jr W H Molecular phylogeny and biogeography of the marine shrimp penaeus Mol. Phylogenet Evol, 1998; 10: 399-407.
4. Barber P.H Palumbi S R Erdmann M. V and Moosa M.K A marine Wallaces's line, Nature, 2000; 406: 692-693.
5. H. Choo, C L, and Liew C Spatial distribution substrate assemblages and size composition of sea horse (*Family Syngnathidae*) in the coastal waters of peninsular Malaysia. Journal of the marine biological association of the United Kingdom, 2003; 83: 227-276.
6. Doherty P. J. Planes S Mather P gene flow and larval duration in seven species of fish from Great Barrier Reef. Ecology, 76: 2373-2391.
7. R. Hall and J D Hollway the plate tectonics of Cenozoic SE Asia and the distribution of land and sea. Biogeography and geological evolution of SE Asia Backhuys publishers Leiden, 1998; 99-131.
8. Irwin D M, Kocher T D Wilson A C Evolution of the cytochrome b gene of mammals Journal of Mol. Evol, 1991; 32: 128-144.
9. Kocher TD., Thomas W. K Meyer A Edwards S V S Villabalance F. X Wilson A C., Dynamics of Mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers Porc Natl. Acad. Sci. USA, 1989; 86: 6916-6200.
10. Lorie S A Pritchard J C Casey s.P Truong S. K Hall H. J and Vincent A C. J. the taxanomy of Vietnam exploited sea horses. Journal of the Linnean Society, 1996; 66: 231-256.
11. Mukai, T Tsuihiji T Sato Morisawa M Mitochondrial DNA divergence in the sea horse Hippocampus conatus (*Syngnathiformes syngnathide*) collected from sagami Bay. Jpn. J., Ichthyol, 2000; 47: 139-143.
12. Nelson J. S Hoddell R J Chou L M Chan W K & Phang VPE Phylogeographic structure of the false clownfish, Amphiprion ocellaris explained by sea level changes on the sunda shelf Marine Biology, 2000; 137: 727-736.
13. Saiki Rk Gelfand D H sto. el S Higuchi R Horn G.T Mullis K B Erlich H A Primer directed enzymatic amplification of DNA with a thermo stable DNA polymerase Science, 1998; 239: 487-491.