DNA BARCODING ANALYSIS OF BRACKISH WATER SHRIMPS FROM CHILIKA LAGOON

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Abstract: Chilika Lake, the biggest brackish water lagoon in Asia, could be a prominent biodiversity hotspot along the Indian geographical region. During last decade DNA barcoding has become a preferred method of choice for molecular specimen identification. Here we present a comprehensive DNA barcode library of shrimp taxa found within the Chilika Lake. During this study, we've provoked molecular data of taxonomically identified blackish water shrimp from the Chilika Lagoon. We characterized three species of shrimp parted into three distinct groups, which are genetically diverse from one another and exhibited identical phylogenetic relationship to their respective genus. Three species were characterized by three BINs. Intraspecific divergences ranged from 0.0 to 0.42% with a mean of 0.20%, while divergences for the species during a genus ranged from 0.00 to 14.3% with a mean of 11.44%.The distances within families ranged from 23.55 to 25.11% with a mean of 24.29%. Application of DNA barcodes as a highly effective identification system for the analyzed crustaceans of the Chilika Lake and represents a very important breakthrough for contemporary biodiversity assessment studies using barcode sequences.

Key words - Shrimps, COI gene, DNA barcoding, phylogenetic relationship.

I. Introduction

Chilika lagoon, the biggest brackish water lagoon of Asia and therefore the second largest within the world, could be a hot spot for biodiversity and harbours rich aquatic flora and fauna and as a listed as Ramsar site in 1981. Geological evidences indicate that the Chilika Lake was a part of the Bay of Bengal during the later stages of the Pleistocene period (Pascoe 1964). The linking of freshwater rivers and stream into the lake form a component freshwater character which allows proliferation of a tremendous number of species diversity. Chilika Lake is bordered between the ocean and mountains. Penaeid shrimp belong to the biggest phylum within the kingdom Animalia, the Arthropoda, which is characterized by the presence of jointed appendages and an exoskeleton or cuticle that's periodically molted. Moreover arthropoda having 47,217 known species of which 3,580 has been already barcoded. (Bouchet 2006).Within the Arthropoda, most DNA barcoding publications focus on insects (Fernanda 2011, Saad 2017, Zhou 2017, Hausmann 2011, Woodcock 2013, Morinière 2014, Raupach 2014) whereas the number of comprehensive studies analyzing the utility of DNA barcodes for the discrimination of crustacean species is still poor (Lefébure 2006, Costa 2007, Radulovici 2009) Nevertheless, crustaceans represent one of the most ecologically and economically important invertebrate groups (Brusca 2003). The foremost abundant Family is Penaeidae having Genus Penaeus and Species: monodon, japonicus, indicus, merguiensis, vannamei (Reddy 1995), Although, *L. vannamei* is native to the tropical East Pacific from the Gulf of California, Mexico to northern Peru; but presently considered one in every of the foremost widely cultured shrimp within the planet (Barnes 1983). *L. vannamei* the most widely cultured shrimp in the world (Liao 2011) and is raised in at least 27 countries, with major production operations occurring in the US, Mexico, Central America, tropical South America, China, India, and southeast Asia. *L. vannamei* has also been introduced to Asia. The first introduction apparently occurred in 1980 in the Philippines, followed by Taiwan in 1981 and mainland China in 1988. In 1996, mainland China and Taiwan started commercial production of *L. vannamei* and from there, aquaculture production spread rapidly throughout the Asia, including Thailand, Indonesia, Vietnam, the Philippines, Malaysia and India (Rosenberry, 2004; Briggs et al., 2004). Asia (particularly China, Thailand and Indonesia) now produces 75% of the worlds *L. vannamei*, with only 25% being produced in its original Western hemisphere. Brackish water ecosystem provides a natural habitat to the Crustacean has six species of penaeid shrimps recorded to the present point (Reddy 1995).viz. *Penaeus monodon, Penaeus semisulcatus, Fenneropenaeus indicus, Metapenaeus* Genus Penaeus having species are the foremost economically important around the globe, monoceros, Metapenaeus affinis, and Metapenaeus dobsoni. The shrimps are ecologically and economically important species as they play a significant role within the ecosystem still as highly traded united of the favourite seafood (Jayachandran 2001). Shrimps production (*Penaeus monodon* and *Penaeus vannamei*) has rapidly increased since the last 20 years. In eighteen century the assembly of shrimps is half thereto of in nineteen century in metric tons (Rath and Dev Roy 2009). Shrimps are a significant a component of the marine food web. Some species of shrimps are cultivated in aquaculture in tropical countries (FAO, 2016). Shrimps contribute about 20% by volume of the world seafood market (Gillett 2008) around 90% of the shrimp's production in farms (*Penaeus monodon* & *Penaeus vannamei*) has been done around the world. (FAO 2004). Generally, over 10 million lots of crustaceans are produced annually for human consumption. Species identification by morphological features is sometime ineffective and misleading, because, larval stages of some species groups often cannot be assigned to the right species (Nicole 2012, Maralit 2013). The morphological identification is more complicated when the species are damaged because of rough handling, and there may have chances for shrimp's fraud (Nicole 2012). The unique colour system in crustacean often plays a extremely important role in aquaculture because their colour affects the standard and value. Prawns, like most other crustaceans are able to change colour depending upon growth, background coloration and time of day due to chromatophores. (Montgomery 2010). These problems are often overcome by DNA Barcoding technology. The concept of DNA barcoding relies on the concept that each species will have similar DNA barcodes representing its intraspecific variability. Additionally, the interspecies variation should exceed the intraspecies variation, which allows a transparent genetic delineation of species; reflect barcoding gaps (Hebert et al., 2003; Hebert et al 2003a, and Hebert et al., 2004). the two main advantage of DNA barcoding are (i) to assign unknown

specimens to already described and classified species, and (ii) to strengthen the invention of latest species and facilitate identification, particularly in cryptic, microscopic, and other organisms with complexity in their morphology (Hebert et al., 2003; Hebert et al., 2003a) Whereas various phenomena may affect the applying of DNA barcodes or mitochondrial DNA normally for successful specimen identification, e.g. heteroplasmy (Doublet et al 2008; Doublet et al; 2013) incomplete lineage sorting (Baeza et al; 2013) the presence of mitochondrial pseudogenes (numts) (Song et al., 2008; Buhay et al., 2009) or introgressive hybridization (Taylor et al., 1993) Barber et al 2012). DNA barcoding has become a awfully important tool in numerous biological disciplines, e.g. modern biodiversity assessment studies (Bucklin et al., 2011; Barber et al., 2006; Leray et al., 2015), conservation biology (Witt et al., 2007) or the authentication of sea food (Haye et al., 2012, Nicole et al., 2012) As consequence, many recently published species descriptions and taxonomic studies included barcode sequence data (Khalaji et al., 2014; Markert et al., 2014). Once a year many penaeid shrimps are traded from this lake, nevertheless, because of several anthropogenic threats, the native population are frequently sick with diseases and loss the value (Rath and Dev Roy 2009).The morphology based species identification and estimates of the variability of penaeid shrimps, is difficult due to their morphological variations in several life stages, phenotypic plasticity and sexual dimorphism [(Prasanna Kumar et al., 2012). Hence, the morphology-based assessment frequently misleads the species identification and thus increases the risks of sea food fraud (Nicole et al., 2012). DNA barcoding is that the applying of short sequences of DNA to species identification and has become a robust discipline since its introduction in 2003 (Hebert et al., 2003a) visiting assess and document biodiversity at a quicker pace than taxonomical methods. Delimit species boundaries and reveal cryptic species within known taxa (Wiens 2007), additionally as barcode analysis for discovery of recent or undescribed species (DeSalle 2006; Rubinoff et al., 2006). Mitochondrial genome of crustaceans was considered to possess numerous advantages over the nuclear genome due to lack of introns, limited exposure to recombination, high copy numbers in every cell, haploid character, a generally strict maternal mode of inheritance, high substitution rates and lack of fast nucleotide substitution within the mitochondrial genome where the marker is found. Mitochondrial DNA (mtDNA) sequences are widely accustomed study genetic variability in aquaculture species including crustaceans and these sequences have proved extremely useful in elucidating genetic variability and phylogenetic relationships among many crustacean(Cunningham et al., 1992; Chu et al., 2003). Barcoding studies can aids to detect overlooked species with complex morphology and enhanced to collection of additional genetic, morphological, ecological and geographical data (Bucklin & Frost 2009, Hebert et al., 2004a, Smith et al., 2008b, and Steinke et al., 2009b) Accurate species identification is critical for understanding their distribution and abundance and to tell ecosystem-based management. It's been effective in a metazoan group, including brackish caridean shrimps.
The fragment of the mitochondrial COI gene appear to be a decent marker for speciation studies and population analysis in Crustaceans (Burkenroad 1983), having length is approximately is 652 bp, the cytochrome oxidase subunit I (COI) gene has been standardized to spot the penaeid shrimps (Rajkumar et al., 2015., Jose et al., 2016; Saad and El-Sadek 2017). Different molecular-based approaches even are tested to spot the commercialized products of shrimps to substantiate their origin (Besbes et al., 2015).So far, several studies were aimed to figure out the range of shrimps from southern a part of India through DNA barcoding approaches (Mamatha et al., 2016; Subbaiya et al., 2017). Hence, the research work first aimed to work out the potential of mitochondrial COI gene to spot the taxonomically identified penaeid shrimps from Chilika Lake and also evaluate their genetic diversity. The generated barcode data would enrich the worldwide database, help to estimate the population structure of morphologically static species and also detect the commercial seafood fraud. This study aims to first develop a comprehensive DNA barcode library for the Shrimps from the Chilika Lake. This could improve the standard of future monitoring programs by linking barcode sequences with carefully identified voucher specimens. This study also will provide a more robust understanding of the genetic variation in shrimps and to provide baseline information for creating improved conservation strategies for this lake ecosystem. Furthermore, the knowledge should be more readily available to non-taxonomists, researchers and policy makers to assist in their efforts to determine effective management of this ecosystem.

II. Methods & Materials

Ethical statement

We declare that, the Shrimps under study are not protected under wildlife conservation act and are routinely caught by professional fisherman and sold as a food in Indian markets. No specific permission is required for collecting these Shrimps in India, and no single experiment has been done on live organism in the laboratory.

Sample collection

Present study examines shrimp species within the portion of the Chilika Lake. The specimens were collected from the market of Balugaon very close to the Chilika Lake (19° 42' 27.72" N 85° 10' 45.48" E) in eastern coast of Odisha state. Most of the shrimp's specimens were digitally photographed, in case of multiple specimens, representative images were used. The collected specimens were identified by available keys (Reddy 1995; Isabel 1997; Dholakia 2010). The specimens were preserved in 95% alcohol and deposited at the Paul Hebert Centre for DNA Barcoding and Biodiversity studies, Dr. Babasaheb Ambedkar Marathwada University Aurangabad, MS, India.

DNA Extraction and PCR amplification

DNA isolation from muscle tissue using Promega wizard genomic kit following the method decribed by Sambrook et al (2005) quantified using Nanodrop spectrophotometer (ND1000, Thermo Corporation, USA) and quality checked on 1% Agarose gel electrophoresis. Gel was visualized using gel documentation system (Bio Rad Inc., USA). DNA diluted to have 100 ng/µl concentrations and stored at -20°C for further mitochondrial marker processing. COI gene amplification was performed in 25 µl PCR reaction using Kappa biosystem PCR kit constitute 12.5 µl of 10 % Trehalose, 5.5 µl Nuclease free water, 2.5 µl 10X PCR buffer, 0.4 µl MgCl2, 2.0 µl dNTPs, 0.5 µl, 10mM Fish primer(Paine et al., 2007; Ward et al., 2005)

FishF2 5′TCGACTAATCATAAAGATATCGGCAC 3′
FishR2 5′ACTTCAGGGTGACCGAAGAATCAGAA 3′
FR1d 5CACCTCAGGGTGTCCGAARAAYCARAA3'

VF2 5'TCAACCAACCACAAAGACATTGGCAC 3'
and 0.1 µl taq polymerase (5 units/µl) and 1.5 µl of 100 ng/µl of template DNA.
The PCR thermal cycle condition includes an initial denaturation step of 2 minutes at 95°C, 35 cycles of 30 sec at 94°C, 40 sec at 52°C and 1 minute at 72°C, with final extension at 72° C for 10 minutes. Amplified PCR products were visualized 0mn 1.5% Agarose Gel.
PCR products were processed for cleanup to remove unincorporated nucleotides and residual primers using 0.25 µl Exonuclease (Exo)(20 units/ µl) and 0.5 µl of Shrimps Alkaline phosphatase (SAP) enzyme (1 unit/µl) followed by cycle sequencing reaction using Big Dye Terminator v3.1 cycle sequencing kit (Applied Biosystem, Inc.) with 16th folds dilution. Using COI gene amplicon sequencing Fish F2/R2 and VF2/FR1d pairs of primers were used. The thermal cycle condition were an initial denaturation of 2 minutes at 96°C and 35 cycles of 30 sec at 96°C, 15 sec at 55°C and 4 minutes at 60°C. The cycle sequencing is followed by sequencing clean up by ethanol precipitation followed by dissolving templates in HiDi Formamide and bidirectional sequenced in ABI 3130 and 3730 Genetic Analyzer. The generated COI sequences are in table 4.

Table 1.Morphological differences identified in brackish shrimp species

Species	Common Name	Rostral Teeth: Upper/Lower	Body Colour	Uropod Colour	Appearance of Telson	Antennal Colour
M. affinis	Jinga Shrimp	7-8/0	Brownish yellow	White with red margin	No distal fixed pair of spines on the telson	Reddish brown
P. monodon	Tiger Shrimp	6-8/3	Grayish, greenish or dark greenish blue; Reddish brown in large adults.	Reddish with black margin	……	Brownish red
M. dobsoni	Kadal Shrimp	5-8/0	Yellowish red	Red	With a pair of distal spine and series of minute	Very long; Yellowish red

Data analysis for COI gene and Species and delimitation

The DNA barcode sequences and the standard associated metadata were uploaded to BOLD systems platform (www.boldsystems.org) (Ratnasingham & Hebert 2007) and assigned to submit to the Barcode of Life Database under the project CHKLS. To analyse the barcode sequence database were used online BOLD tools: Distance Summary, Barcode Gap Analysis and Barcode Index Number System (BIN). To illustrate the phylogenetic arrangement of species and groups, we generated a dendrogram through Neighbor-Joining reconstruction under Kimura 2-parameters (K2P) model using MEGA v. 6.06. (Kimura 1980; Tamura et al., 2013) The statistical robustness of the branches was evaluated by bootstrap test with 1000 pseudo-replicates.

Data analysis

Compared the sequence from each specimen with barcode sequences on GenBank using 'Blast' (Altschul et al., 1990) and with sequences on BOLD using the 'Identification Request' function. Using the barcode gap criterion, a species is distinct from its nearest neighbour (NN) if its maximum intraspecific distance is less than the distance to its NN sequence. The 'Barcode Gap Analysis' (BGA) was performed using BOLD. Species identification success by 'Best Match' and cluster analysis was performed using Taxon DNA (Meier 2008). Genetic divergences increased with taxonomic rank (Table 3 ;) with little overlap between conspecific and congeneric distances. Intraspecific divergences ranged from 0.0 to 0.42% with a mean K2P genetic distance between species was 0.20%, 11.44% between species within genus and 24.29% between genera within family (Table 2). The average K2P genetic distance within genus was 57 fold higher than average K2P genetic distance within species.

Table: 2 - Mean percentage base composition and GC content of the first, second, and third codon positions from 10 specimens belongs to 3 species.

	Min	Mean	Max	SE
G %	17.8	18.46	19.7	0.1919
C %	19.25	21.38	23.27	0.5729
A %	27.14	27.54	27.96	0.076
T %	30.47	32.62	35.61	0.5882
GC %	37.06	39.84	41.87	0.6025
GC % Codon Pos 1	49.07	53.25	56.74	0.8804
GC % Codon Pos2	42.36	43.05	43.78	0.132
GC % Codon Pos3	18.75	23.2	26.73	0.9667

III. Result

Taxon diversity

A total 10 specimens of shrimps belonging to three species, 2 genera and 1 family collected at the 1 site. We generated 10 COI sequences for 3 species. All amplified sequences were >500 bp (mean, 625 bp) with no insertions, deletions stop codons and NUMTS.

COI diversity assessment

All 10 specimens were successfully sequenced generating 3 haplotypes. The sequence read lengths were 620 bp with average nucleotide composition of A=27.54%, T=32.62, G= 18.46%, C=21.38% in table 2. The base composition showed that AT content (60.16%) was more than the GC content (39.84%). The mean T content was the best, and also the mean G content was rock bottom. The GC content decrease within the order of first, second, from the third codon position with mean of 53.25%, 43.05% and 23.0% respectively. All the COI sequence, no insertion, deletion or stop codon were detected.

Table: 3 - Percentage K2P sequence divergence at the COI barcode within and between various taxonomic levels based on the studied Shrimps species from Chilika Lake, India.

Label	n	Taxa	Camparison	MinDist (%)	MeanDist (%)	Max Dist (%)
Within Species	10	4	8	0.00	0.20	0.42
Within Genus	7	1	16	0.00	11.44	18.97
Within Family	10	1	21	23.55	24.29	25.11

Table 4:- COI DNA Barcode generated from the shrimp species from Chilika Lake.

Species	COI sequence	bp
M. monoceros	ATCATTCGAGCTGAACTAGGTCAACCAGGTAGTTTAATTGGAGACGATCAAATTTATAATGTCGTA GTTACTGCCCACGCTTTCGTTATAATTTTCTTTATAGTTATGCCAATTATAATTGGAGGATTCGGTA ACTGACTAGTCCCTCTTATACTTGGTGCCCCAGATATGGCATTCCCACGAATGAATAATATAAGAT TCTGACTTCTCCCCCCTTCTCTAACTCTCTTACTTTCAAGAGGAATAGTAGAAAGAGGAGTAGGAA CGGGATGAACAGTTTACCCCCCTCTAGCAGCAGGAATTGCTCATGCTGGAGCTTCAGTTGATATAG GAATTTTCTCGCTACACCTTGCAGGAGTCTCATCAATCTTAGGAGCAGTTAATTTCATGACAACAG TTATTAATATGCGCCCTGCAGGAATAACTATAGACCGTATACCACTCTTCGTATGAGCGGTCTTTA TCACAGCCTTGCTACTATTACTATCCCTCCCAGTCCTAGCCGGAGCAATCACTATATTGCTAACTG ACCGAAACCTTAATACTTCATTCTTTGACCCAGCGGGTGGTGGAGACCCCATCCTTTATCAACATT TATTTTGATTCTT	608
P.vannamei	TCCGAGCTGAATTAGGTCAACCTGGGAGCCTCATTGGGGATGATCAAATTTATAACGTAGTTGTCA CAGCTCACGCTTTTGTAATAATTTTTTTATAGTTATACCAATTATAATTGGAGGATTTGGTAATTG ACTAGTACCTTTAATGTTAGGTGCCCCAGATATAGCCTTCCCTCGAATGAATAATATAAGCTTCTG GTTATTACCTCCTTCTCTTACATTGCTTTTATCAAGAGGAATGGTTGAAAGAGGTGTCGGAACCGG ATGAACGGTATACCCTCCTTTATCTGCCAGTATTGCTCACGCTGGAGCTTCAGTAGATCTTGGAAT TTTCTCTCTTCACTTAGCTGGAGTATCTTCTATTCTGGGAGCAGTAAACTTTATAACAACTGTAATC AATATACGATCTACAGGAATAACTATAGACCGTATACCTCTATTTGTATGAGCAGTATTTATCACT GCTTTATTACTACTTTTATCATTACCAGTCTTAGCAGGAGCTATTACTATACTTTTAACAGACCGTA ATCTTAACACATCATTCTTCGACCCAGCAGGAGGAGGAGACCCAGTTTTATATCAACATTTATTCT GATT	601
M.dobsoni	GTTTAACCAAACCACAAAAGACATTGGAACTTTATATTTTATTTTCGGAGCTTGGGCTGGTATAGT AGGTACAGCTTTAAGTTTAATTATCCGAGCTGAGTTAGGTCAACCAGGTAGATTAATTGGGGACG ATCAGATTTATAATGTTGTAGTCACTGCCCACGCTTTTGTTATAATTTTCTTTATAGTTATACCAAT CATAATTGGAGGGTTTGGTAATTGACTTGTTCCCTTAATGCTTGGTGCCCCAGATATAGCGTTCCC ACGAATGAATAATATGAGTTTTTGATTACTTCCTCCTTCATTAACCCTTTTACTTTCAAGAGGAATA GTAGAAAGAGGAGTGGGGACAGGATGAACAGTATACCCTCCTCTAGCAGCAGGAATTGCCCATG CAGGTGCCTCAGTTGACATAGGAATCTTTTCTTTACATTTGGCTGGAGTTTCATCAATCCTAGGAG CAGTTAATTTCATAACAACAGTAATCAACATGCGACCTGCTGGAATAACTATAGACCGAATACCA CTTTTTGTTTGAGCAGTTTTTATTACGGCACTGCTCCTTTTACTCTCACTTCCTGTGCTTGCAGGAG CAATTACTATACTATTAACAGACCGAAATCTCAATACAACTTTTTTCGACCCAGCAGG	651

Table 5: List of the studied Shrimps species from Chilika Lake and their BOLD accession numbers.

Class	Order	Family	Barcode Index Number	Species
Malacostraca	Decapoda	Penaeidae	BOLD:ADK2293	*Metapenaeus monoceros*(n=5)
Malacostraca	Decapoda	Penaeidae	BOLD:AAD7619	*Penaeus vannamei*(n=3)
Malacostraca	Decapoda	Penaeidae	BOLD:ACQ9665	*Metapenaeus dobsoni*(n=2)

Notes: n is number of sequences.

Estimation of evolutionary divergence between sequences

The number of base substitutions per site from between sequences is shown. Analyses were conducted using the Kimura 2-parameter model. This analysis involved 10 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for every sequence pair (pairwise deletion option). There has been a complete of 678 positions within the final dataset. Evolutionary analyses were conducted in MEGA5.1

Species delimitation

The assessment of species identities with already known sequence and closely related species in BLAST and BOLD databases gives 98%-100% identities indicating the potential of COI sequence to produce species level identification. Additionally, Barcoding Gap Analysis showed that every one species had a maximum intraspecies distance of but 2%. Further, the NJ phylogenetic tree showed that each one the recognized species forms monophyletic clusters with none overlap between species by providing 1000 bootstrap for resolving congeneric samples. Species with two or more barcode sequences were analyzed for species identification using Taxon DNA. When a third threshold was employed, 100% of the species were correctly identified using the most effective close match criteria. Latter analysis also showed that all specimens exhibited high distance values to their nearest neighbour indicating the presence of “barcode gap” among the 3 observed species, in table 6.

Table 6: the mean and maximum value for each species, compared to the nearest neighbor distance

Sr. No.	Species	Mean Intra-Sp	Max Intra-Sp	Nearest Species	Distance to NN
1	*M. dobsoni*	0.19	0.19	*M. mononceros*	17.55
2	*M. monoceros*	0.2	0.42	*M. monoceros*	17.55
4	*P. vannamei*	0.13	0.21	*M. mononceros*	23.55

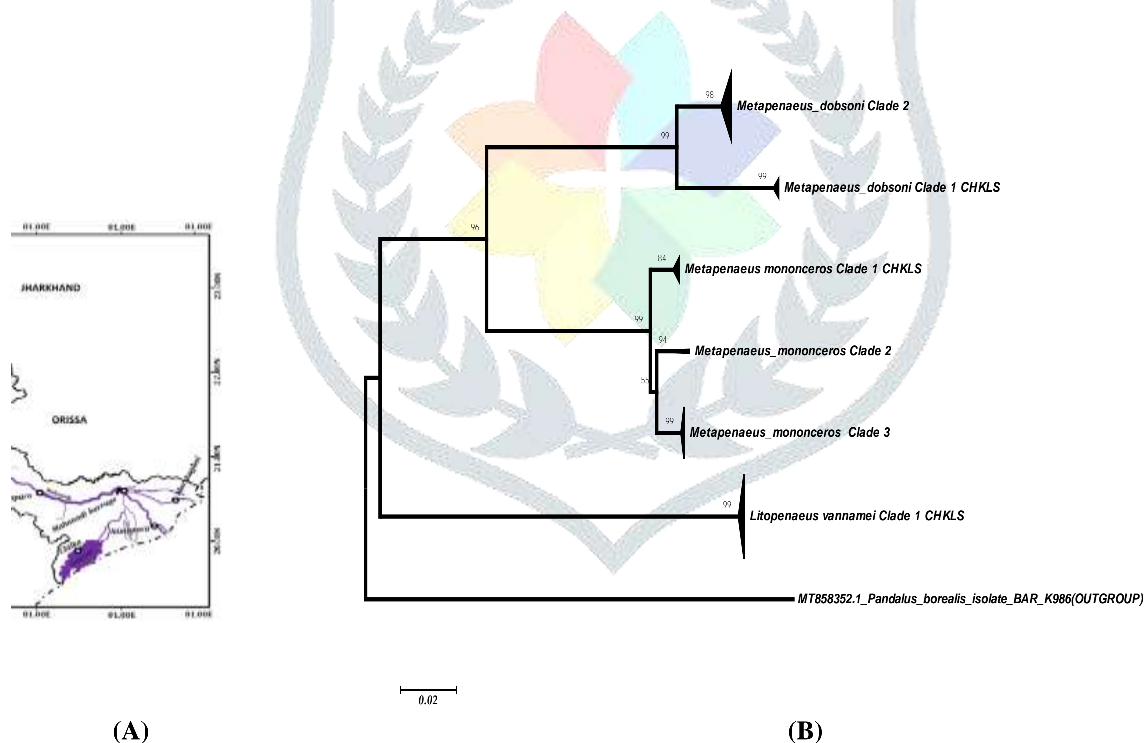

Fig 1: (A) Collection site of shrimp Indicated by arrow of the studied shrimps species from Chilika Lake. (B) Neighbour-joining (NJ) tree of the studied shrimps with 1000 bootstrap support. caridean shrimp used as an out-group in the phylogeny. Clade 1 CHLKS show the species in the present study.

IV. Discussion

Besides, the invasion of non-native gene pool may possess threat to the indigenous penaeid shrimp In the present dataset, the *Metapenaeus dobsoni* shows two clades in NJ tree, one clade (*Metapenaeus dobsoni* CHKLS Clade-I) consisting of two sequences; generated from present study. However, the remaining 49 sequences were generated from Mozambican coastal waters, Chilika Lake,

Indian coastal in addition as Maharashtra coastal line. The *Metapenaeus monoceros* Clade 1 CHKLS form separate clade apart from Clade 2 and Clade 3 it predict divergence in between them, its might be a separate population. Further the *M. monoceros* and *M. ensis* often possess difficulties in species-level identification thanks to overlapping morphological. The current study identified two specimens as M. ensis, however, the molecular data resulted as *M. monoceros* in NJ tree. The 2 specimens show distinct clade with high bootstrap support and sufficient genetic divergence with M. monoceros. Moreover the generated sequences of *M. monoceros*, collected from Chilika Lake resulted distinct clade in NJ tree and high genetic divergence, in fig 1. The study revealed that the *M. monoceros* population of Chilika Lake may represent isolated gene pools, which require to be re-examine completely. The genetic variability of shrimp species are tested by COI gene and also the observed variations were correlated to the geographical isolation. (Vergamini et al., 2011; Rossi and Mantelatto 2013). Perhaps mtDNA evolution is accelerated in shrimp, or perhaps stabilizing selection over a protracted fundamental quantity has reduced rates of morphological change. Thus, to observe and protect the native species during this eco-system and forestall the ingress of exotic taxa, both morphological and DNA based species assessment is also adopted eventually. Cluster analysis revealed that each one 3 species examined within the study formed a monophyletic cluster which corresponded perfectly with the taxa recognized on morphological criteria. Although three species pairs showed limited divergence (<3%), maximum intraspecific divergence was always under the NN distance, enabling the separation of all species. Analysis of genetic variability and geographic differentiation of such organisms is important for the event of effective resource management programs. This sort of knowledge is required for maintaining and improving the culture and management efficiency of *P. monodon* (Carvalho and Hauser 1994; Ward and Grew 1994). The DNA barcoding considered to be a tool to spot, invent and study specimens so as to know the range of species within an ecosystem and also to gauge the genetic variability within species. The present study, the universal vertebtrate primers FishF2 and FishR2, VF2 and FR1d worked fine to amplify a couple of 650 bp region of the COI gene from 3 Shrimps species of the family Penaeidae without stop codons, insertions or deletions.

Barcode Gap Analysis' showed that NN distance for all the species was above the utmost intraspecific distance. The Barcode index (BIN) system provided further evidence of the genetic distinctiveness of the species because it assigned the 4 species to 4 BINs. When identity analysis was performed using Best Match/Best Close Match at a third threshold, all the species were correctly identified (Meier 2008). Our study confirms that employing COI barcoding can help within the identification of the bulk of shrimp's species in diverse lake systems. Increasing use of DNA barcoding can overcome the constraints of morphology based identifications and help identify previously unidentified species by documenting the variety of COI sequences within currently recognized species. This use of molecular data should be complementary to morphological analsysis in such endeavours, and also the establishment of reliable global COI barcode databases for shrimps will help to be able to accurately identify any shrimps at any stage of the life cycle (such as eggs or larva) (Trivedi et al., 2011) or maybe from small pieces of tissue. This may be a valuable tool within the hands of ecologists and aquaculture conservators. In future generating barcode sequence data for large biodiversity collections is provided by metabarcoding where the amplification and sequencing of pooled community samples (Yu, D. W. et al., 2012)

V. CONCLUSION

DNA barcoding proved to be a useful tool for the identification of brackish shrimps of Chilika Lake and highlights an approach that allows a given ecosystem at minimal cost and effort to evaluate the genetic divergence of cryptic species. Our results clearly underline the usefulness of DNA barcodes to discriminate the vast majority of the analyzed species. It is evident that the mtCOI is a successful partial gene segment to construct the phylogeny of crustacean species. Thus, the resulted high-genetic divergence between the two clades in the dataset might depict two different populations of M. mononceros and M. dobsoni. Further it needs more extensive sampling from different region within the Chilika Lake and generation of more DNA barcode data of studied species from different geographical localities to resolve the genetic differences.

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