Swimming crab of family portunidae rafinesque-schmaltz

Science, Geography

Swimming crab of family portunidae rafinesque-schmaltz 1815 are crustaceans inhabit sub tidal estuarine and offshore waters and widely distributed across the indo-west pacific region. charybdis de haan 1833 is the second largest genus within the sub-family thalamitinae paulson 1875. mitochondrial dna 16s rrna gene was used to evaluate the genetic variation of c. feriata in indo pacific region. for the analysis the sequences procured from pakistan coupled through therepresentative gene sequences of india china vietnam in addition taiwan obtained from genbank. The genetic heterogeneity was estimated as nucleotide diversity and haplotype diversity whereas the result indicated that there was no genetic differentiation among the representatives of c. feriata in indo pacific region. test of neutrality was revealed that c. feriata might have undergone a population expansion event. this preliminary research contributing a slight measure in better understanding the genetic variation of c. feriata in indopacific region and provide a valuable insight for the proper conservation and management of this highly valued species in coastal waters of Pakistan.

Introduction

Subfamily thalamitinae (Paulson, 1875) the largest family o family portunidae comprising one hundred and fifty-three species belonging to four genera charybdisgonioinfradens thalamita and thalamitoides. according to ng et al. 2008 charybdis de haan 1833 is the second largest genus with sixty species distributed among 4 sub genera charybdis de haan 1833 goniohellenus alcock1899 gonioneptunus ortmann 1893 goniosupradens leene 1938 charybdisferiata linnaeus 1758 crustacea: decapoda: portunidae are common inhabitants of tidal estuarine and offshore waters and are

widely distributed across the indo pacific region includes south and east africa gulf of oman arabian gulf pakistan bay of bengal india sri lanka indonesia singapore vietnam china hong kong taiwan philippines thailand malay peninsula japan and australia. c. feriataeasily distinguished by its grayish-brown background cream and orange brownbands and a central cross-shaped pattern or patches on the gastric region of the carapace. in the arabian gulf it is rare uncommon in bombay coast indiaquite prevalent in coast of pakistan. its immense size of 181 110 mm tirmizi and kazmi 1996 makes an opportune edible species and export to foreign countries by the frozen condition and frequently sold fresh or frozen in local markets. in pakistan it commands substantially higher premium price being sold for 150-250 rs per kg.

Besides their commercial significance some taxonomic work has been done in the indo pacific region including pakistan milne edwards 1861; hashmi 1963; chhapgar 1957; khan 1975; yu 1976; sakai 1976; wee and ng, 1995; apel and spiridonov 1998; Stephenson, 1967; tirmizi and kazmi 1996; neumannand spiridonov 1999; ng et al. 2008 some studies focused on the coi huang 2009 microsatellite markers ma et al. 2013 rapd jin et al. 2004 16s rrna zhang et al. 2008 and complete genome study ma et al. 2015 although c. feriata seems to quite prevalent in fisheries catch from the coastal waters of pakistan in the last few years the stock of c. feriata has been declined the same situation has also been observed by josileen 2008 and recognized it to the over-exploitation as well as environmental deterioration. the present study has been expressed valuable insight into the genetic variation in

charybdis feriata linnaeus1758 in indo-west pacific waters including Pakistan for the proper conservation and management of this species for this purpose the genetic discrepancy in present study expressed as inferred from 16s rrna mitochondrial dna.

Materials and methods

Specimens of charybdis feriata linnaeus 1758 were purchased from the Karachi fish Harbour Pakistan figure 1 and stored at -20°c for further molecular analyses. In total five specimens of charybdis feriata selected for the study. Chela muscles was used for the extraction of the genomic DNA the process have been done by the qiagens dneasy blood and tissue kit cat. no. 69504 a 523-base pair bp of the target dna segments was amplified by the polymerase chain reaction pcr using two universal primers of 16srrna gene in the mtdna genome. Polymerase chain reaction was completed in an applied biosystem 2720 thermal cycler. thermal cycling profile was carried out with following steps: denaturation (initial) cycle fof 10 min at 95ºc followed by 40 cycles of 1 min at 95ºc 1 min at 46ºc and 2 min at 72ºc with a final extension of 72ºc for 10 min and the primers 16sar 5′-cgc ctgttt atc aaa aac at-3′ paired with the reverse primer 16sbr 5′-ccg gtc tga act cag atc acg t-3′ palumbi et al. 1991; schubert et al. 2000; fratini et al. 2005 successfully amplified pcr products were sent to the macrogen company koreafor purification and sequencing. dna sequences data were analyzed through applied bio system sequence scanner v1. 0 software. a new sequence data were submitted to the genbank. dna sequences were initially searched for sequence similarity based on blasting 2. 2. 26+ zhang et al. 2000 the new

nucleotide sequences have been submitted to the ncbi nucleotide-sequence databases ku296934 ku296935 ku296936 ku296937 and ku130125

In addition the following sequences archived in molecular databases were included in our analyses: genbank accession no: kf 3861471 ay 497291. 1 dq062727. 1 china am 410535. 1 vietnam and kj 132522. 1 taiwan the obtained dna sequences were initially aligned using clustal w thompson et al. 1994 evolutionary relationship was analyzed by using the maximum likelihood method according to tamura-nei model tamura and nei 1993 evolutionary analysis was directed using mega6: molecular evolutionary genetics analysis version 6. 0 tamura et al. 2013 the procured dna sequence data were utilized to calculate the genetic heterogeneity within c. feriata and estimated as the haplotypes h and nucleotide diversity pi the neutrality tests fus fs fu 1997 and tajimas 1989 d-test was applied to test the deviations from neutral molecular evolution by using software dna sp version 5. 10 librado and rozas 2009

Results and discussion

The amplicon of 16s rrna gene of charybdisferiata from found inl Pakistani coastal waters showed 99% highest homology with the the representative of c. feriata representative of india china vietnam and also taiwan. Additionally all available partial 16S rRNA fragment of c. feriata acquired from gene bank accession no: kf 220503. 1-kf 220506. 1 kf 386147. 1 india kf 3861471 ay 497291. 1 dq062727. 1 china am 410535. 1 vietnam and kj132522. 1 taiwan for the further studies. All available data of indo pacific region (n= 14) was estimated for the evolutionary phylogenetic tree construction and verify the

genetic relationship in dissimilar Indo pacific population whereas chaybdis annulata worked as out-group. the evolutionary history was contingent by using the maximum likelihood method based on the tamura-nei model tamura-nei 1993 by (NJ) neighbor-joining method a matrix of pairwise distances expected with the help of maximum composite likelihood approach. The phylogenetic analysis involved 14 nucleotide sequences and a whole 299 positions in the final dataset. the result revealed the monophyletic relationship in all respective sequences of c. feriata from india china vietnam taiwan and pakistan.

The haplotype was estimated in c. feriata from pakistan five representatives was selected out of which three haplotype were obtained and the haplotype diversity was 0. 70 whereas the as indo pacific region haplotype diversity was was revealed that diversity was 1. 0 in india 0. 667 in china whereas 1. 0 in vietnam and taiwan. the nucleotide diversity pi indicates the percent difference among the haplotypes in species. Nucleotide diversity was highest in india 0. 0113 and the lowest was observed in china 0. 0014 whereas the nucleotide diversity 0. 00163 observed in pakistan table 1 the genetic heterogeneity was shown that there was no phylogenetic discrimination within the the representatives of c. feriata in indo pacific region. ma et al. 2015 and huang 2009 noticed that haplotype diversity ranged between 0. 867-0. 891 in the complete genome of c. feriata and 0. 787 in coi gene. These were quite similar toward the present study. fratini et al. 2002 and lai et al. 2010 revealed that the moderately high haplotype diversity and relatively minimum sequence divergence approximately less than 0. 5% in

portunid crabs similar with other marine crustacean or marine organisms with planktonic larvae. Similarly according to zhou et al. 2016 within the sesarmid crab was showed moderate level of hap diversity h and a low-level of nucleotide divergence ranged from 0. 338 – 0. 731 and from 0. 00058 – 0. 00278 respectively. the high genetic divergence attributed the dissimilar environmental conditions features of life individual span population size. These factors help in maintaining a high level of divergence in thepopulations nei 1987; avise 2000; ma et al. 2015 ma et al. 2015 also observed nucleotide divergence in charybdis feriata in multiiple areas on the southeast coast of china and ranged between 0. 0011-0. 0013 per locality that was very similar to the current study.

An extensive distribution has been predictable intended for many marine species belonging to different taxa supporting the idea that speciation is a rare event in the marine realm becker et al. 2007 neutrality tests were applied to estimation of neutral evolution tajimas d that revealed d= -1. 48074 p 0. 10 whereas fu and lis was d =-1. 82689 p 0. 10 fus and fs statistics was d=-1. 475 p 0. 10 suggest either population expensive or purify selection. in an indo pacific region the d statistics was negative with a significant deviation from mutation-drift equilibrium and suggested population expansion or purify selection table 2 according to ma et al. 2015 c. feriata underwent population expansion after a period of low effective population size and sudden population extension can affect population genetic divergence and haplotype. in population expansion event due to the process of mutation further haplotypes were produced but then was

removed by genetic drift avise et al. 1984 c. feriata have been facedhigh exploitation pressure and their catch gradually decline in the last few years and there is a need for proper protection and organization of this species.