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Survey, Morphometric and Molecular Analysis of Marine Crab Charybd is Feriatus (Linnaeus, 1758) from Southeast Coast of India

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ABSTRACT

In population studies, Morphometric and molecular investigation provides a powerful complement to environmental and genetic stock identification approach and length-weight relationships allow the conversion of growth-in-length equations to growth-in-weight for use to a stock assessment. The survey of C. feriatus were made in eight landing centers, viz., Annankoil, Mudasalodai, Arukkattuthurai, Muthupettai, Mallipattinam, Sethubavchatram, Manamelkudi and Jegathapattinam, Southeast coast of India. Whereas other study animals were collect only from Annankoil(Lat.11°24' N; Long. 79°464' E). In general, male crab landings contributions were highest followed by females irrespective of the stations of the current study. C. feriatus males showed to undertake positive allometric growth at value of 'b' being 3.06. C. feriatus females showed to undergo negative allometric growth in 'b' value of 2.89. The COX1 Sequenced nucleotide regions were deposit in GenBank under the accession number KR186011. The present tree topology clearly shows that the C. feriatus are close similarity with next species of C. japonica, C. hellerii and C. lucifera in neighbor-Joining method.

Keywords: *Marine crab; C. feriatus, Population studies, morphometric, molecular analysis*

INTRODUCTION

Relatively minimum is known about life history of the flower crab, *C. feriatus*, from Indian waters (Ramesh babu *et al*., 2002a, 2006; John Samuel *et al*., 2004; Dinesh babu, 2011; Sound arapandian *et al.*, 2013; Gyanaranjan Dash *et al.*, 2014). The length-weight relationship thus yields information on the general well-being of individuals, variation in growth according to sex, size at first maturity, gonadal development, and breeding season. Study on the length-weight association in aquatic animals has large application in delineate the growth patterns throughout their developmental pathways (Bagenal, 1978).

In population studies, morphometric analysis provides a influential complement to environmental and genetic stock identification approach (Cadrin, 2000) and length-weight relationships permit the conversion of growth-in-length equations to growth-in-weight for apply in stock assessment model (Moutopolos and Stergiou, 2002). Information about individual body weight-length/ width relationships in populations is significant for estimate the population size of a stock, specifically for the purpose of its exploitation. The length-width/ weigh relationships are regard suitable for evaluating crustacean populations (Atar and Sector, 2003; Gorce *et al*., 2006; Sangun *et al*., 2009).

Sequence data from the Cytochrome Oxidase I (COI) gene support the hypothesis of Dall *et al.* (1990). A/T base compositional bias was present in the first and third codon positions. This bias is stable to that reported for other crustaceans to be a lot of exaggerated (Crease, 1999). DNA sequences were determined for portions of the mtCOX 1 and provide evidence of the lack of genetic distinction of species, distinct species, and point out the clear need for further extensive and intensive ecological and genetic analysis (Kozol *et al*., 2012). Hence the present study was aimed to assess the *C.feriatus* fishery resources from Annankoil to Jegathapattinam in southeast

coast of India for a period of two years, Morphometrics: length-weight relationship and molecular identification were used to confirmation the animal at species level and stock assessment.

MATERIALS AND METHODS

Survey

In Tamil Nadu (Lat. 13°09' N and Long. 80°27' E) coast, the fishery data was collected from 8 landing centers *viz.*, Annankoil, Mudasalodai, Arukkattuthurai, Muthupettai, Mallipattinam, Sethubavchatram, Manamelkudi and Jegathapattinam. The study was made from April 2013 to March 2015. In this study, healthy individuals of male and female were collected (John Samuel *et al.*, 2004). For the purpose of total crab catch estimation; the observation of crab landings and counting of baskets were taken into consideration. Each and every station was visited twice in every week and observations were recorded. Similarly, the details for non-sampling fishing days were collected from the merchant's diary (crab marketing agency) for as many days as possible. The average daily crab landing was worked out from the data thus obtained and raised to the number of fishing days to assess the monthly total crab landings of the centre. Data was not collected during fishing banned period/holiday (April end and May).

Statistical Analysis (SPSS Package)

One way analysis of variance was done for the data to crab fishery of *C.feriatus*. The level of significance was seen at 5% level ($p>0.05$).

Identification of C. feriatus

For the study, samples of *C.feriatus* were collected from Annankoil (*Lat.*11°24′ *N*; *Long.* 79°464′ *E*), along the southeast coast of India.

Morphometrics and length-weight relationship

Regression equations were calculated assuming an allometric growth equation ($Y = a + bX$), to determine relations between different morphometric characters in males and females. The values of the correlation coefficient (r) were calculated to know the pattern of association between propodus/abdomen and carapace dimensions (Snedecor and Cochran, 1967), with the objective of establishing a mathematical relationship between the variables, so that if one variable is known, the other could be computed approximately.

The carapace width-weight relationship was estimated using the log form of the allometric growth equation W= aLb (Pauly, 1983a), where W =expected weight, L = total carapace width, 'a' = *y*-intercept or the initial growth coefficient, and 'b' = the slope or growth coefficient. The values of constants of 'a' and 'b' were calculated by the least squares method. The differences in the carapace width-weight relationship between sexes were tested by ANOVA (MS Excel). A total of 500 males and 350 females were used for the study.

Molecular identification

Genomic DNA was isolated from the soft tissues using the Extraction Kit procedure NucleoSpin® Tissue Kit (Macherey-Nagel) following manufacturer's instructions. The quality of isolated DNA was checked using agarose gel electrophoresis. Polymerase chain reaction (PCR) amplification reactions were carried out in a PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems). PCR cycling parameters included an initial denaturation phase at 97°C for 10 min, followed by 40 cycles at 94°C for 1 min, 48.5°C for 1 min, and 72°C for 2 min, and ended with a final extension at 72°C for 13 min. Amplified products were visualized in agarose gels (2%) stained with Ethidium bromide and purified using the kit Wizard® SV gel. Sequencing reaction was done in a PCR thermal cycler using the Big Dye Terminator v3.1 Cycle sequencing Kit. Cytochrome c Oxidase Subunit I were amplified using universal primers Forward (dgLCO) 5'-GGTCAACAAATCATAAAGAY ATYGG-3' Reverse (dgHCO) 5'-TAAACT TCAGGG TGACCAAARAAYCA-3' delineate by Meyer (2003).

Amplified products were visualized in a UV transilluminator (Genei) and the image was captured under UV light using Gel documentation system (Bio-Rad). COXI sequences were edited with ProSeq 2.91 and multiple alignments were done using ClustalW. The probability of substitutional saturation for the COX1 was determined statistically using DAMBE 4.5.56 (Xia &Xie 2001). Sequence alignment and required editing of the obtained sequences were carried out using Geneious Pro v5.1 (Drummond *et al.*, 2010). The phylogenitic tree was constructed using MEGA6 (Tamura *et al.*, 2013).

RESULTS

Survey

The study period was divided into four seasons viz., summer (April, May and June), premonsoon (July, August and September), monsoon (October, November and December) and post monsoon (January, February and March). The male and female crabs of *C.feriatus* in different stations are presented in tables 1-5. In the present study, Annankoil and Mudasalodai male

and female crabs were uniformly landed maximum in the month of January and minimum in the month of November. Arukkattuthurai and Muthupettai male and female crabs were landed maximum in the month of January and minimum in the month of August. Mallipattinam and Sethubavchatram male and female crabs were landed in plenty during the month of January and minimum in the month of November and July. Manamelkudi and Jegathapattinam male and female crabs were landed maximum in the month of January and minimum in the month of July and August (Tables 1-4).

Table1. *The male crab landings (Kg) of C. feriatus in different landing centers of South East coast of Tamil Nadu from August 2013 to July 2014*

Stations	Months (Kg)											
	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	June	Jul	Total
Annankoil	286	267	248	217	269	771	421	397	352	243	251	3722
Mudasalodai	291	295	278	252	294	829	325	431	420	374	285	4074
Arukkattuthurai	97	132	129	142	127	287	179	125	117	98	94	1527
Muthupettai	321	342	357	346	351	891	342	411	435	395	326	4517
Mallipattinam	489	476	487	465	498	943	532	556	521	443	476	5886
Sethubavchatram	398	365	376	385	397	932	416	421	385	393	311	4779
Manamelkudi	532	524	511	523	521	949	614	585	463	396	373	5991
Jegathapattinam	432	453	467	421	532	898	525	502	486	407	454	5577
Total	2846	2854	2853	2751	2989	6500	3354	3428	3179	2749	2570	**36,073**

**May -fishing holidays*

Table2. *The female crab landings (Kg) of C.feriatus in different landing centers of South East coast of Tamil Nadu from August 2013 to July 2014*

Stations	Months (Kg)											
	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	June	Jul	Total
Annankoil	120	119	121	132	145	342	187	167	142	121	97	1693
Mudasalodai	134	121	112	138	153	451	194	171	163	141	113	1891
Arukkattuthurai	18	21	33	37	77	86	72	44	39	28	26	481
Muthupettai	153	162	153	148	163	532	231	185	153	132	115	2127
Mallipattinam	234	243	274	294	405	706	497	432	323	289	206	3903
Sethubavchatram	171	173	163	185	196	597	212	203	174	139	131	2344
Manamelkudi	245	246	286	306	426	749	523	436	358	303	238	4116
Jegathapattinam	218	204	265	285	396	637	476	395	264	232	254	3626
Total	1293	1289	1407	1525	1961	4100	2392	2033	1616	1385	1180	**20,181**

**May -fishing holidays*

Table3. *The male crab landings (Kg) of C.feriatus in different landing centers of South East coast of Tamil Nadu from August 2014 to July 2015*

Stations	Months (Kg)											
	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	June	Jul	Total
Annankoil	293	283	262	215	273	842	428	414	363	221	296	3890
Mudasalodai	298	284	292	264	320	858	416	461	435	421	301	4350
Arukkattuthurai	95	141	135	152	136	304	185	134	113	88	96	1579
Muthupettai	326	331	361	321	366	883	363	431	416	383	331	4512
Mallipattinam	471	487	491	452	503	962	521	541	537	451	463	5879
Sethubavchatram	392	351	366	367	405	897	364	431	342	363	362	4685
Manamelkudi	541	515	531	514	541	923	587	598	494	447	494	6185
Jegathapattinam	416	448	474	432	545	936	563	520	414	416	423	5587
Total	2832	2840	2912	2744	3089	6605	3427	3530	3114	2790	2766	**36,667**

**May -fishing holidays*

Table4. *The female crab landings (Kg) of C.feriatus in different landing centers of South East coast of Tamil Nadu from August 2014 to July 2015*

Stations	Months (Kg)											
	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	June	Jul	Total
Annankoil	148	185	156	147	179	562	216	210	186	163	121	2273
Mudasalodai	147	153	157	162	196	589	210	296	164	162	142	2378

Arukkattuthurai	42	54	35	42	84	120	98	60	71	36	42	684
Muthupettai	184	193	179	159	198	647	297	198	186	158	149	2548
Mallipattinam	273	262	296	307	452	731	523	461	322	315	244	4186
Sethubavchatram	190	192	172	204	205	626	248	258	185	191	164	2635
Manamelkudi	262	251	292	371	489	783	489	451	362	391	264	4411
Jegathapattinam	271	236	225	298	403	683	546	341	231	264	275	3773
Total	2817	1526	1987	1690	2206	4741	2627	2275	1707	1680	1401	**22,888**

**May -fishing holidays*

Total crab landings

The total landing of *C. feriatus* from eight stations in 2013-2014 and 2014-2015 were recorded to be 56,254 and 59,555 Kg respectively. Among eight stations, the overall total crabs landed were maximum in Manamelkudi and minimum in Arukkattuthurai. The crab landing was comparatively high in Aug 2014 to Jul 2015 (59,555 Kg) than that of Aug 2013 to Jul 2014 (56,254 Kg) (Table 5)**.** The total landings of the crabs were observed in the following order during 2013-2014 and 2014-2015; Manamelkudi (10107 and 10596 kg.) > Mallipattinam (9789 and 10065 kg.) > Jegathapattinam (9203 and 9360 kg.) > Sethubavchatram (7123 and 7320 kg.) > Muthupettai (6644 and 7060 kg.) > Mudasalodai (5965 and 6728 kg.) > Annankoil (5415 and 6163 kg.) > Arukkattuthurai (5965 and 6728 kg) (Table 4).

The total male crab landings in Manamelkudi (5991 and 6185 kg.) was maximum followed by Mallipattinam (5886 and 5879 kg.), Jegathapattinam (5577 and 5587 kg.), Sethubavchatram (4779 and 4685 kg.), Muthupettai (4517 and 4512 kg.), Mudasalodai (4074 and 4350 kg.), Annankoil (3722 and 3890 kg.) and Arukkattuthurai (1527 and 1579 kg.) in 2013-14 and 2014-15 (Tables 1 & 3). In female crab landings; Manamelkudi (4116 and 4411 kg) was ranking first followed by Mallipattinam (3903 and 4186 kg), Jegathapattinam (3626 and 3773 kg), Sethubavchatram (2344 and 2635 kg), Muthupettai (2127 and 2548 kg), Mudasalodai (1891 and 2378 kg), Annankoil (1693 and 2273 kg) and Arukkattuthurai (481 and 684 kg) (Tables 2 & 4) in 2013-14 and 2014-15. In general, male contributions were maximum followed by females irrespective of the stations of the present study. The crabs landed in all landing centers showed (Table 5).

Table5. *Total (Kg) male and female crabs of C.feriatus in different landing centers of South East coast of Tamil Nadu from August 2013 to July 2015*

Stations	Aug 2013 to Jul 2014 (Kg)			Aug 2014 to Jul 2015 (Kg)		
	Male	Female	Total	Male	Female	Total
Annankoil	3722	1693	5415	3890	2273	6163
Mudasalodai	4074	1891	5965	4350	2378	6728
Arukkattuthurai	1527	481	2008	1579	684	2263
Muthupettai	4517	2127	6644	4512	2548	7060
Mallipattinam	5886	3903	9789	5879	4186	10065
Sethubavchatram	4779	2344	7123	4685	2635	7320
Manamelkudi	5991	4116	10107	6185	4411	10596
Jegathapattinam	5577	3626	9203	5587	3773	9360
Total	36,073	20,181	**56,254**	36,667	22,888	**59,555**

Table6. *One way analysis of variance for the crabs landed at different landing centers of South East coast of Tamil Nadu from August 2013 to July 2014 and August 2014 to July 2015*

Source of Variation	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	887240.626	7	126748.661	9.748	.000
Within Groups	897153.374	69	13002.223		
Total	1784394.000	76			

Length – weight relationship

In this study *C. feriatus* males showed to undertake positive allometric growth at value of 'b' being 3.06 (Fig.1). C. feriatus females showed to undergo negative allometric growth in 'b' value of 2.89 (Fig. 2).

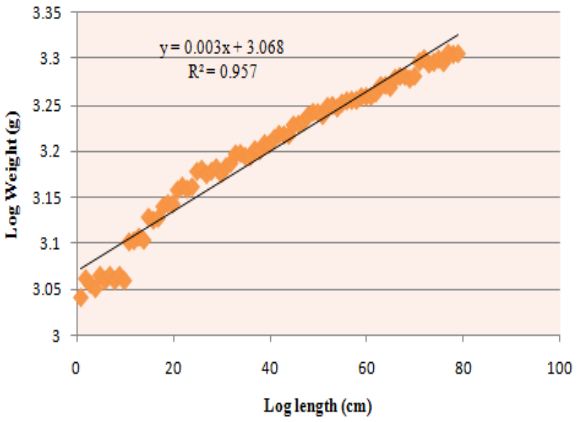

Fig1. Length – weight relationship of male *C.feriatus*

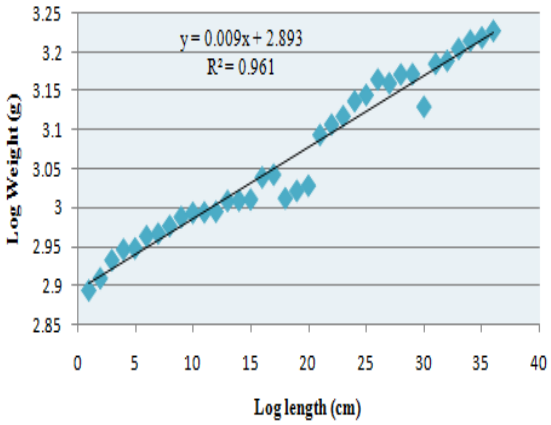

Fig2. Length – weight relationship of female *C.feriatus*

Molecular identification

Isolation of genomic DNA

The genomic DNA was isolated from the soft tissues C. feriatus. DNA isolated was checked for its purity by agarose gel electrophoresis. The gels were visualized in a UV transilluminator (Genei) and the image was captured under UV light using Gel documentation system (Bio-Rad) (Fig.3).

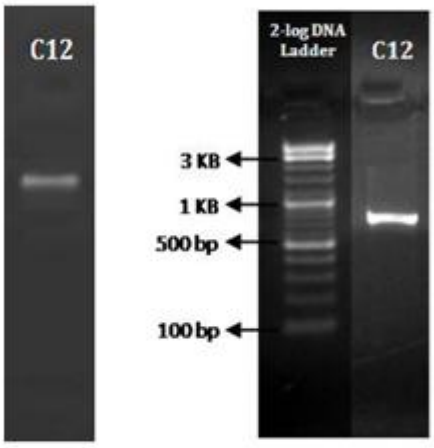

Fig3. Total genomic DNA extracted and PCR amplified COX 1 gene from *C. feriatus*

PCR amplification of Mitochondrial Cytochrome Oxidase I (COX 1) gene

The figure showed PCR amplification of COX 1 gene compared with standard molecular marker (approximately 700 bp length) (Fig.3). The COX 1 Sequenced nucleotide regions were deposited in GenBank under the accession number KR186011.

Blast

Based on the performance of BLAST program, it was confirmed that sequences were new to NCBI – Genbank. In addition, all sequences were showed maximum (up to 99%) degree of similarity with its closely relatives. The obtained sequences were used to construct the phylogenetic tree and genetic distance between the species (Fig.4).

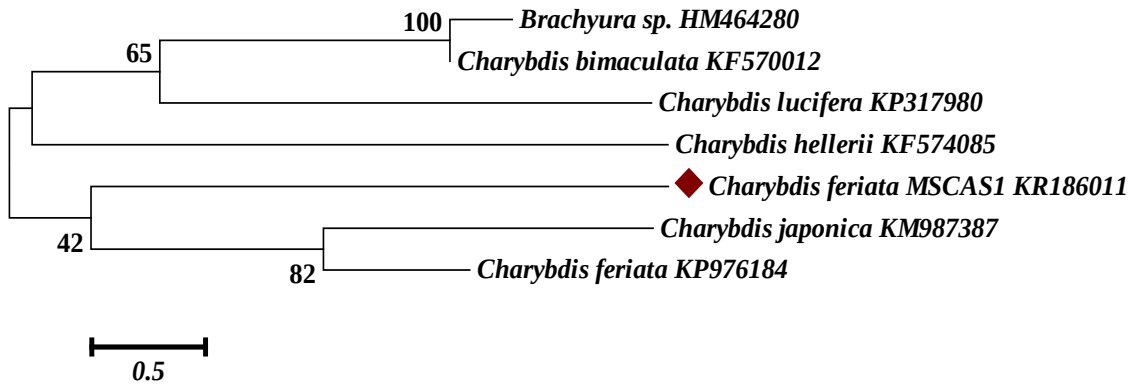

Fig4. *Evolutionary relationship of marine crab species using neighbor-joining method*

Phylogenetic analysis

The evolutionary history was inferred using the neighbor joining method and the combined data from the *C. feriatus* species – groups were analyzed to produce a dendrogram and it was shown in Fig. 4, Table 7. The present tree

topology clearly shows that the *C. feriatus* are close similarity with next species of C. *japonica*, *C. hellerii* and *C. lucifera* in neighbor-Joining method.

Table7. *Mean pairwise sequence distances comparisons based on K2P (in percentage) between the analyzed taxa*

S.No	Species	1	2	3	4	5
1	C. feriatus MSCAS1 KR186011	***				
2	Brachyura sp. HM464280	5.09142	***			
3	C. japonica KM987387	3.97541	5.78619	***		
4	C. hellerii KF574085	6.13994	4.26104	6.03928	***	
5	C. lucifera KP317980	4.91203	3.53264	6.35198	5.91992	***
6	C. bimaculata KF570012	4.96667	0.17111	4.64506	4.37361	3.51147
7	C. feriatus KP976184	5.23324	4.61218	2.09097	3.90490	4.95811

DISCUSSION

As in the present study, commercially important crab's landings were already reported by different authors (Radhakrishnan, 1979; John Samuel *et al.*, 2004; Varadharajan *et al.*, 2009; Varadharajan, 2012; Ilavarasan *et al.*, 2015). The crab landing varied in the past two decades are due to many reasons (CMFRI, 2009). Landing is abundant in some areas in india is due to large number of trawl operations. The important mechanized fishing centers are Mallipattinam, Jegathapattinam, Mudasalodai and Sethubavchatram but in other station the fisherman using only the fiber boat, catamaran and small boats (CMFRI, 2010).

The present study report by total male crab landings in Manamelkudi (5991 and 6185 kg.) was maximum followed by Mallipattinam (5886 and 5879 kg.), Jegathapattinam (5577 and 5587 kg.), Sethubavchatram (4779 and 4685 kg.), Muthupettai (4517 and 4512 kg.), Mudasalodai (4074 and 4350 kg.), Annankoil (3722 and 3890 kg.) and Arukkattuthurai (1527 and 1579 kg.) in 2013-14 and 2014-15 (Tables 1 & 3). In female crab landings; Manamelkudi (4116 and 4411 kg) was ranking first followed by Mallipattinam (3903 and 4186 kg), Jegathapattinam (3626 and 3773 kg), Sethubavchatram (2344 and 2635 kg), Muthupettai (2127 and 2548 kg), Mudasalodai (1891 and 2378 kg), Annankoil (1693 and 2273 kg) and Arukkattuthurai (481 and 684 kg) (Tables 1 & 3) in 2013-14 and 2014-15. In general, male contributions were maximum followed by females respective of the stations.

The previous report by Ilavarasan *et al.* (2015) also supported the present findings among four landing centers; *C.lucifera* was maximum in Mudasalodai and minimum in Pudukuppam. A similar trend was already reported by Soundarapandian *et al.* (2013) for *P. vigli*. The present study, *C.feriatus* was slightly higher than that reported by Varadharajan, (2012).

In this study report *C. feriatus* males showed to undertake positive allometric growth at value of 'b' being 3.06. *C. feriatus* females showed to undergo negative allometric growth in 'b' value of 2.89

C. feriatus is a small, fast growing tropical crab species having higher natural mortality, because it tends to be unrealistically high for small tropical species with high natural mortality (Gulland, 1983; Pauly and Munro 1984).The value of exponent 'b' was found to be 3.06, 2.86 (length-weight relationship) male and female respectively. The relationships between carapace width and weight and carapace length and weight have many uses. They are, for example, indicators of condition, and are used to calculate biomass and to estimate the recovery of edible meat from crabs of various sizes (Lagler, 1968). Even though the change of b values depends primarily on the shape and fatness of the species, various factors may be responsible for the differences in parameters of the width weight relationships among seasons and years, such as temperature, salinity, food (quantity, quality and size), sex, and time of year and stage of maturity (Pauly, 1984; Sparre, 1992).

Further the 'b' values indicated that the males are heavier than females at a given width and length against weight in *C.feriatus*. The linear plot based on the calculated values suggested that there is a direct relationship in length-weight relationships in males and females of the study animals. In the present analysis 'b' values were very much closed to 3.0 and therefore, *C. feriatus* does follow the cube law (LeCren, 1951; Martin, 1949). It should be noted that the allometric coefficient *b* in the relationship between carapace width and crab weight lies between 2.80 and 3.2, that is, within the range most

commonly reported for other Baltic crustaceans (Normant *et al.,* 2000). Besides the statistically significantly greater individual weight for the same carapace width in males, sexual dimorphism is obvious, especially in the size of the pincers (Jesse 2001, Czerniejewski *et al.* 2003). Padayatti (1990) also observed that males grow faster and grows to bigger size than females.

The carapace width and length, claw length, propodus width and abdominal length and width reported a linear relationship which appears, therefore that the energy gained by these crabs from ingested foods gets evenly distributed in the body building processes as also reported in *T. gigas* (Vijayakumar *et al.,* 2000).

The linear regressions between crab length and weight were highly significant. The carapace length-weight relationships were allometric for both sexes. There were no significant differences in slopes between males and females.

The present tree topology clearly shows that the *C. feriatus* are close similarity with next species of *C. japonica, C. hellerii* and *C. lucifera* in neighbor-Joining method.

Crabs are difficult to identify with morphological methods. *C.feriatus* collected from Annan koil, South east coast was identified at their molecular level using COI sequences. In this study, the sequenced data were analyzed to understand the phylogenetic inter-relationship within the Tunicata (Folmer *et al.,* 1994). The BLAST results provided confirmation of our taxonomic identifications for all examined species. This high number of parsimony-informative sites indicates that COI mtDNA is capable to be an informative locus candidate for phylogenetic studies (Kamarudin *et al.,* 2011). Thorpe *et al.,* (2001) reported that the phylogenetic analysis was done to confirm the identification of invertebrates in its different developmental stages. Thus, molecular methods are the very perfect way of identifying crab species.

CONCLUSION

In all landing centers the male and female crabs were uniformly landed, maximum in the month of January and minimum in the month of August. Total crab landings of *C. feriatus* in Tamil Nadu coast was 25,342Kg. Among eight stations in Tamil Nadu coast the overall total crabs landed was maximum in Manamelkudi and minimum in Arukkattuthurai. In this study female *C. feriatus* showed to undergo positive allometric growth and males are negative allometric growth. Its helps to understand the crabs body growth and weight. Molecular taxonomic studies based on analysis of COX 1 nucleotide sequences revealed the study species belongs to *C. feriatus*. The crab specie *C. feriatus* was identified through mtCOX1 gene sequence which confirmed by phylogenetic tree topology. The species need to study with more number of coding genes (12S and 16S) with different ecosystem might confirm the gene flow and evolutionary status of crabs species.

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