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Morphological analysis and molecular phylogeny of *Aristeus alcocki* Ramadan, 1938 from south-west coast of India

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Present study has combined both morphometric as well as molecular phylogenetic analysis to describe *Aristeus alcocki* on the basis of the specimens procured off Sakthikulangara along the south west coast of India. Morphometric analysis of *A. alcocki* was confirmed by the relative length of the chela/carpus ratio as P1: 1.14-1.35, P2: 0.95-1.07, P3: 0.7-0.85. Pleurobranchia of pereopod I-IV in the form of minute papillae without pinnules. Hepatic groove well formed straight and anterior part slightly curved. Strong buttress with well developed pterygostomian spine anteriorly curved. Molecular phylogeny of the species was analyzed using two nuclear-protein coding genes, phosphoenolpyruvate carboxykinase (PEPCK) and sodium–potassium ATPase α-subunit (NaK) and two mitochondrial genes, large subunit ribosomal DNA (16S rDNA) and Cytochrome oxidase I (COI). These four highly conserved genes were sequenced and phylogenetic analysis was performed. From this study the specimen was identified as *A. alcocki* and the molecular data submitted in GenBank will be a contribution towards identification of the species using molecular tools in future.

[**Key words**: Deep-sea shrimps, Morphology, Molecular phylogenetics, Taxonomy]

Introduction

Molecular phylogenetics is a novel tool which combines molecular and statistical techniques to infer evolutionary relationships among organisms. Molecular phylogenetics uses the structure and function of molecules and how they change over time to infer these evolutionary relationships[1]. It is a powerful tool to detect uniqueness of individuals, populations or species based on their molecular data[2,3]. It can give precise information on evolutionary relationship of the individual species avoiding taxonomic ambiguities[4-6]. The nucleotide sequence difference within a gene reflects the evolutionary relationship between two organisms. Specific DNA regions on nuclear or mitochondrial genes will act as useful information resources required for species identification[7]. Molecular phylogenetics has become very common nowadays as whole genome sequencing for complex organisms is faster and less expensive. Advanced computer technology and public availability of genomic data have made molecular phylogenetics to grow further and find newer applications.

Mitochondrial genes are increasingly used in population and phylogenetic studies of marine organisms[8]. Large subunit ribosomal DNA (16S rDNA) gene and Cytochrome oxidase I (COI) genes from mitochondria were used to analyze taxonomic and phylogenetic relationships among many marine crustaceans[9]. Since mitochondrial genes are maternally inherited, the phylogenies derived from them may not provide the entire picture of the evolutionary relationship[10]. Hence, two nuclear protein-coding genes, phosphoenolpyruvate carboxykinase (PEPCK) and sodium–potassium ATPase α-subunit (NaK) are currently being used as efficient molecular information resources for species identification[11]. These two genes are involved in fundamental cellular functions and are highly conserved throughout evolution. They are successfully used to resolve the deep-level phylogeny of organisms[12,13].

Among the penaeoid shrimps, all the species falling under the family Aristeidae are all deep-sea forms occupying the upper continental slope. In Indian waters as many as fifteen species of this family are known to occur of which 3 species are majorly reported as *Aristeus alcocki* Ramadan, 1938, *Aristeus semidentatus* Bate, 1881 and *Aristaeomorpha wood-masoni* Calman, 1925 in the Indian waters at depths upto 3200m[14]. Among these *A. alcocki* is of commercial importance along the south west coast of India[15,16]. A perusal of literature shows that there exist doubts and uncertainties about the true identity of this species from Indian waters[17]. *A. alcocki* is known as Arabian red shrimp because of dark red colour in carapace and abdomen due to high carotenoid

content and it is locally called as 'Red ring' due to the presence of dark red rings along its abdominal pleura.

This study was aimed to construct nuclear and mitochondrial DNA molecular phylogeny based on sequences derived from 16S rDNA, COI, PEPCK and NaK and develop hypotheses on speciation along with information obtained from morophological examination. Based on the morphological as well as molecular investigation this study reports the occurrence of *A. alcocki* in trawls off Sakthikulangara from southwest coast of India.

Materials and Methods

Agarose, Ethedium bromide, Tris(hydroxymethyl)aminomethane, Boric acid, EDTA (Ethylenediaminetetraacetic acid), glycerol and Bromophenol blue were purchased from Merck specialities pvt. ltd (India). Primers for 16S rDNA, COI, PEPCK and NaK were purchased from Sigma Aldrich (St. Louis, Mo, USA). All other chemicals were locally purchased.

Deep-sea shrimp forms a seasonal fishery covering for only about 6 months in the year from January to March and October to December. Samples were collected from Sakthikulangara (lat 8°56'60.78"N long: 76°32'34.27"E) where fishing off Ponnani and Kallamukku fishing harbours (lat: 9°59'02.91"N long: 76°14'33.14"E) i.e., off Cochin during 2013 for carrying out this study (Fig. 1). Samples were obtained from the trawl gear operated at a depth of 400-500 m for 30-60 minutes at a speed of 3-4 knots. Samples were transferred in 100% ethanol to the laboratory in an insulated ice box for further studies. Voucher specimens were deposited in sample collections at Crustacean Fisheries Division, Central Marine Fisheries Research Institute, Cochin, India (CMFRI: CFD: AA3, AA4).

The morphological analysis of the specimens was performed using conventional methods. Samples were procured from the fishing harbour and sex was determined for each shrimp under the binocular microscope, observing the petasma and theylcum. Total length (TL) was measured to the nearest 0.1 mm as the shortest distance between the tip of rostrum to the tip of telson with a digital caliper (precision 0.01 mm) and wet weight (W) with an analytical balance (Saratorius: precision of 0.01 g). The length-weight relationship of *A. alcocki* was calculated as W=aLb (Le Cren, 1951) for both male and female separately and difference in the slopes of the regression lines was recorded. Length (log-transformed) relationships for both male and female were determined by regression analysis (SAS Version 9.3). In order to test "b" value against the isometric value of "3", student's t-test was employed to predict any significant deviation. The t-statistic was calculated as follows: t=(b-3)/Sb where, Sb = standard error of 'b'. Since, a simple linear regression did not give a good fit; a linearised allometric (natural logs) model was used. Model: logY=a+b*logX where X is the independent variable, Y is the dependent variable, representing the measurements of body parts; a and b are constants. Standard assumptions of the regression model (independence and lognormality of residuals, homogeneity of variance) were evaluated using scatter plots of variables, studentized residuals vs. estimates, normality plot of residuals. Rejection levels were defined as statiscally significant ($0.01<P<0.05$) or highly significant ($P<0.01$). Specimens were identified by using traditional morphometric and meristic characters[14,17-21] and finally confirmed by Dr. T.Y. Chan, Ph.D, Professor and Director, National Taiwan Ocean University, R.O.C from the images. The specimens were observed (4X-10X) under Motic image plus 2.0 stereozoom microscope (Motic, Hong Kong) and photographs were taken for all the taxonomic descriptions.

Fig. 1. The geographic location of the landing of sample

Total genomic DNA was extracted from pleopod of the individual specimen preserved under 100% ethanol using DNeasy® Blood &

Tissue Kit (Qiagen Inc.) according to manufacturer's protocol with some modifications. The cells were lysed by incubating at 56°C for 2 h and all other steps were followed as per the protocol. The isolated DNA was stored at -20°C.

Two regions each of the mitochondrial genome, 16S rDNA and COI were amplified, using universal primers 5'-CGCCTGTTTATCAAAAACAT-3' (F), 5'-CCGGTCTGAACTCAGATCACGT-3' (R) and 5'-GGTCAACAAATCATAAAGATATTGG-3' (F), 5'-TAAACTTCAGGGTGACCAAAAAATCA-3' (R) respectively[22,23]. Reactions were performed in 25 µl reaction cocktails containing 0.5 µg/µl genomic DNA, 0.05 U/µl Taq DNA polymerase, 1X buffer, 3 mM $MgCl_2$, 10 pM/µl of each primer and 200 µM dNTPs. The PCR thermal profile used was 94°C for 5 min for initial denaturation, followed by 35 cycles of 94°C for 1 min, 52°C for 1 min, 72°C for 1.5 min, and a final extension at 72°C for 5 min. The PCR products were electrophoresed on a 1.5% agarose gel containing ethidium bromide, and visualized under UV transilluminator (Lark, India).

Two regions each of nuclear genome, PEPCK and NaK were amplified, using universal primers 5'-GCAAGACCAACCTGGCCATGATGAC-R' (F), 5'-CGGGYCTCCATGCTSAGCCARTG-3' (R) and 5'-GTGTTCCTCATTGGTATCATTGT-3' (F), 5'-ATAGGGTGATCTCCAGTRACCAT-3' (R) respectively[10]. Reactions were performed in 25 µl reaction cocktails containing 0.5 µg/µl genomic DNA, 0.05 U/µl Taq DNA polymerase, 1X buffer , 3 mM $MgCl_2$, 10 pM/µl of each primer and 200µM of dNTPs. The PCR thermal profile used was 94°C for 5 min for initial denaturation, followed by 35 cycles of 94°C for 1 min, 55°C for 1 min, 72°C for 1.5 min, and a final extension at 72°C for 5 min for PEPCK. All the conditions were same for the amplification of NaK except for annealing temperature (51°C for 1 min) and $MgCl_2$ concentration (2.5 mM). PCR products were electrophoresed on a 1.5 % agarose gel containing ethidium bromide, and visualized under UV transilluminator (Lark, India).

Amplified PCR products were purified with XcelGen DNA Gel/PCR Purification Mini Kit (Xcelris Labs Limited, India) according to manufacturer's protocol. The eluted PCR products were sequenced directly.

The PCR purified products were sequenced by dideoxy chain termination method[24] using the Big-Dye Ready-Reaction kit v3.1 (Applied Biosystems) on an ABI Prism 3770 automated sequencer from Scigenome, India. The sequence assemblies and contig editions were performed with DNA Baser Sequence Assembler v.4.7 (Heracle BioSoft S.R.L., Romania). The gene sequences obtained were deposited in GenBank and aligned using ClustalX[25] to confirm their identities.

The nucleotide sequences of four gene fragments were aligned with sequences from GenBank by means of multiple sequence alignment using ClustalW algorithm. Phylogenetic trees were inferred for individual loci using the statistical method maximum parsimony with 1000 bootstrap replicates by using MEGA5 software. Maximum parsimony tree was obtained using the Subtree-Pruning-Regrafting (SPR) algorithm[26]. All positions containing gaps and missing data were eliminated.

Results and Discussion

The total length (TL), and weight (Wt) were recorded for all the specimens obtained during the period of observation. During this study the size ranges for TL, Wt varied in male and female from 69-100 mm, 1.6-8.2 g and 86-199 mm, 4.6-22.0 g, respectively. In *A. alcocki* the values for elevation (a) and slope (b) was found to be 0.064, 1.92 and 0.032, 2.19 with their corresponding regression coefficient (r2) of 0.652 and 0.632 in case of male and female, respectively. Length-weight relationship has vital importance in fisheries science.

It helps in establishing mathematical relationship between the two variables, enables conversion of one variable to other to determine possible differences among different stocks of the same species and this method is widely accepted which is less variable and easily measured[27-29]. In the present study the higher value of slope was observed in female compared to male indicating positive allometric growth. The use of total length to determine length-weight morphometric relationships has been widely applied for wild and captive studies as well as other penaeids[30,31]. Parameters of length (TL)-weight (Wt) relationships estimated in the

present study are well within the ranges previously reported for several studies (Figs. 2 and 3).

Aristeu alcocki has a glabrous integument and polished body; postrostral carina is extending beyond the gastric region. Eyes are large and eyestalks has a small tubercle on its inner side, rostrum in female is long and slender with 3 strong teeth on the dorsal side with its anterior part bending downwards till the distal end of antennular peduncle while the posterior part strongly upwards. Rostrum in male is short with its anterior part being bit straight and posterior part surpassed upwards. Upper antennular flagella is compressed sub-foliaceous, extremely short and raised near the base of terminal joint of the peduncle; while the innrer (lower) one is cylindrical and extremely long and springs from the apex of the terminal joint of the peduncle. Antennal scale large, its outer edge strong and acute anteriorly. Antennal flagellum very long mandibular palp is foliaceous with moderate length. Strong buttress with well developed pterygostomian spine anteriorly curved while the antennal spine very small. Hepatic groove well formed straight and anterior part slightly curved. No hepatic spine. Branchiocardiac carina well formed. The first 3 pairs of thoracic legs are chelate with fingers long and slender. Length of chela in 1st pereopod, 2nd pereopod is 1.14-1.35 times and 0.95-1.07 times longer than carpus except in 3rd pereopod where carpus is 0.7-0.85 times longer than chela. Minute movable spine was observed on the anterior part of the merus on its 1st and 2nd pereopod. Last two legs (4th and 5th pereopods) are slender and monodactylus. Pleurobranchs of pereopod1 I-IV (Table 1) are having the form of minute papillae without pinnules. First pair of peleopod without endopod while in male there is subtriangular petasma or andricum. Simple, membranous, right and heft halves united along midline.; glabrous on both

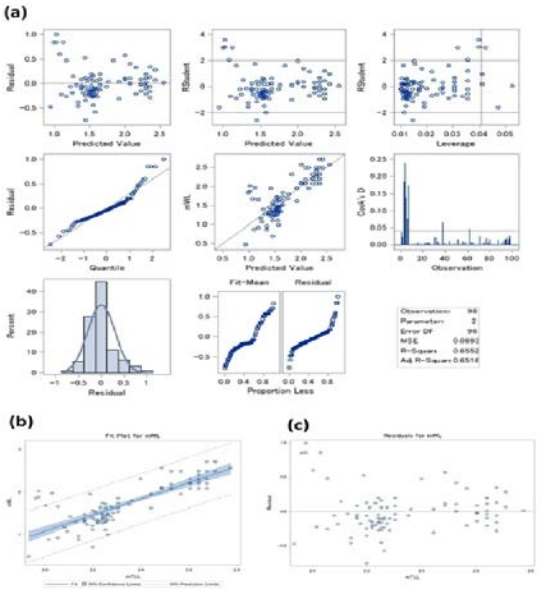

Fig. 2. (a) Fit Diagnostics for LW (b) Fit Plot for LW and (c) Residuals for LW of male specimen of *A. alcocki*

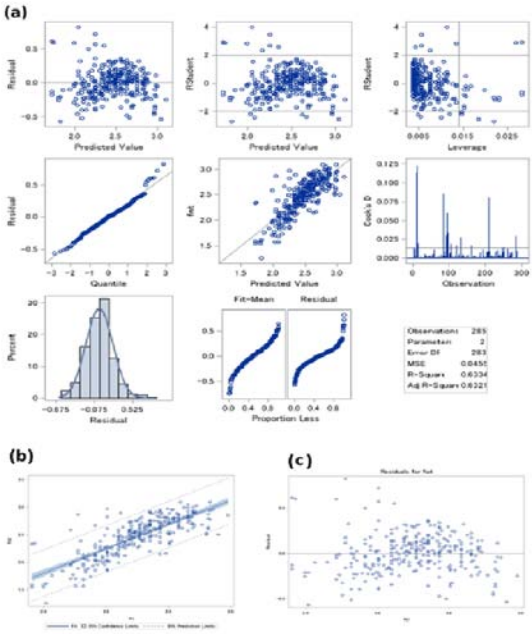

Fig. 3. (a) Fit Diagnostics for LW (b) Fit Plot for LW and (c) Residuals for LW of female specimen of *A. alcocki*

Table 1. Distribution of gills, epipodites and exopodites in *A. alcocki* (M-Maxilliped, P-pereopod)

	M I	*MII*	*MIII*	*PI*	*PII*	*PIII*	*PIV*	*PV*
Epipodites	1	1	1	1	1	1	1	-
Arthrobranchs	1	1	2	2	2	2	2	-
Podobranchs	-	1	1	1	1	-	-	-
Pleurobranchs	-	-	-	1(p)	1(p)	1(p)	1(p)	1
Exopodites	1	1	1	-	-	-	-	-

Table 2. Distribution of photopores on pereopods of *A. alcocki*

	Carpus	*Propodus*	*Dactylus*
Pereopod I	12-16	4-5	0
Pereopod II	9-15	3-4	0
Pereopod III	8-10	3-4	0
Pereopod IV	18-24	20-29	0
Pereopod V	21-26	28-34	9-17

dorsal and ventral surfaces; ventromediam lobules narrow; slightly shorter than lateral lobes and pointed distilly; ventrolateral lobules slightly thicker than dorsolaterial lobules; proximal base thick and medially bends to form short blunt dorsolateral lobule. Appendix maxculina; subrectangular; distal and distolateral margins are have short setae and convex. Appendix interna thin and completely obscured by appendix masculine from dorsal view. Thelycum is a simple open structure. Anterior portion consists of transverse plate arising from sternite between fourth pair of pereopods. Apex slightly rounded, posterior surface smooth consisting of two lateral plates and slightly concave; anterior surface convex and covered with setae. Photopores present on carpus and propodus of all pereopods (Table 2). Red minute dots are present in allover body. 3rd abdominal tergum rounded, the last three abdominal segments are sharply carinate and end in spines. 6th tergum posterior bottom minute spine is present. Telson shorter than inner uropod with 4 pairs of movable spines and uropod have thickend[14,17,18,20,21,32,33]. The specimen closely agrees with the description of *A. alcocki* of Ramadan (1938) in all the above mentioned features (Figs. 4 and 5). George (1966) recorded both *A. alcocki* and *A. semidentatus* from the southwest coast of India but Suseelan (1989) confirmed the presence of only *A. alcocki* in Indian waters by morphological examination. The study has shown that molecular results are in concordance with morphometric results.

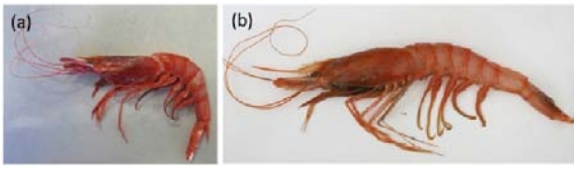

Fig. 4. (a) Male and (b) Female specimen of *A. alcocki*

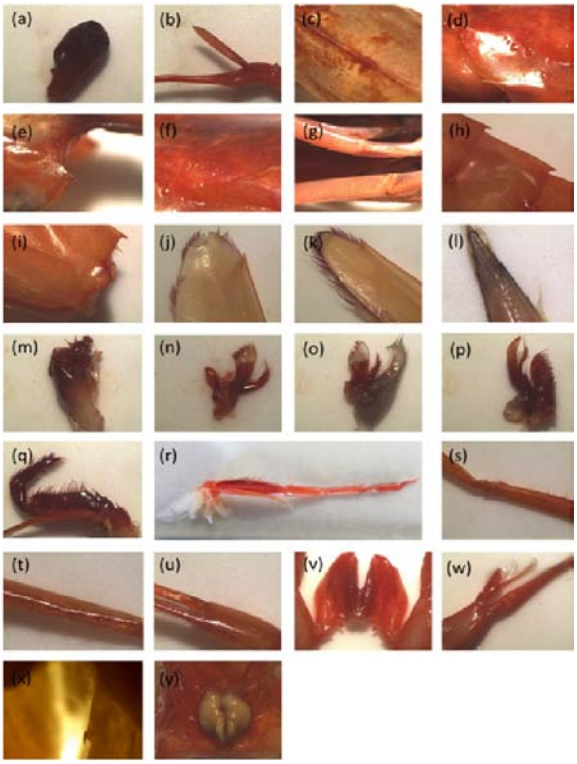

Fig. 5. Morphological description of *A. alcocki* specimen (a) Eye tubercle; (b) Antennular flagella (upper); (c) Postrostral carina; (d) Pterygostomain spine; (e) Antennal spine; (f) Hepatic groove; (g) Red minute dots; (h) 4th and 5th abdominal pluera with posterior spines; (i) 6th abdominal pluera with posterior and ventral spines; (j) Antennal scale outer edge (spine like); (k) Uropod outer edge (spine like); (l) Telson with 4 pairs of spines; (m) Mandibular palp (n) Maxilla 1; (o) Maxilla 2; (p) Maxilliped 1; (q) Maxilliped 2; (r) Maxilliped 3; (s) 1st and 2nd pereopod of merus anterior spine; (t) Photopores on carpus; (u) Photopores on chela; (v) Petasma; (w) Appendix maxculina; (x) Pluerobranchia of minute papillae; (y) thelycum

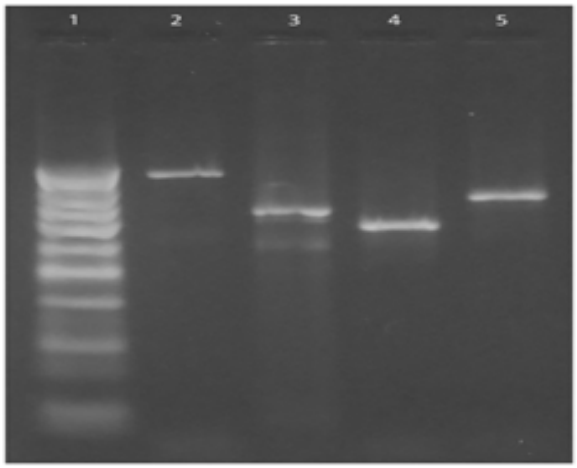

Fig. 7. Agarose gel (1.5%) with Lane 1: 100bp ladder, Lane 2: NaK, Lane 3: PEPCK, Lane 4: 16S rDNA and Lane 5: COI.

In order to analyse the specimen on molecular terms, this study was undertaken. The total genomic DNA from the pleopod of the specimen was isolated and specific mitochondrial and nuclear genes were PCR amplified. Gel picture of the PCR amplified 16S rDNA, COI, PEPCK and NaK showed that they are in the size range of approximately 570 bp, 750 bp, 700 bp and 900 bp respectively (Fig. 7). The PCR amplified gene fragments were sequenced and the sequences were submitted in GenBank with accession numbers (Table 3).

Table 3. Nucleotide base composition of gene fragments of the specimen sample

Gene fragment	Accession No.	Length (bp)	A	T	G	C	GC content (%)
16S rDNA (Isolate 1)	KJ396316	537	158	182	123	74	36.68
16S rDNA (Isolate 2)	KJ486492	331	96	116	76	43	35.95
COI (Isolate 1)	KJ396317	592	147	202	119	124	41.04
COI (Isolate 2)	KJ486493	456	118	156	93	89	39.91
PEPCK (Isolate 1)	KJ396318	534	127	110	136	161	55.61
PEPCK (Isolate 2)	KJ486494	458	110	91	120	137	56.11
NaK (Isolate 1)	KJ396319	582	155	137	149	141	49.82
NaK (Isolate 2)	KJ486495	582	154	137	149	142	50

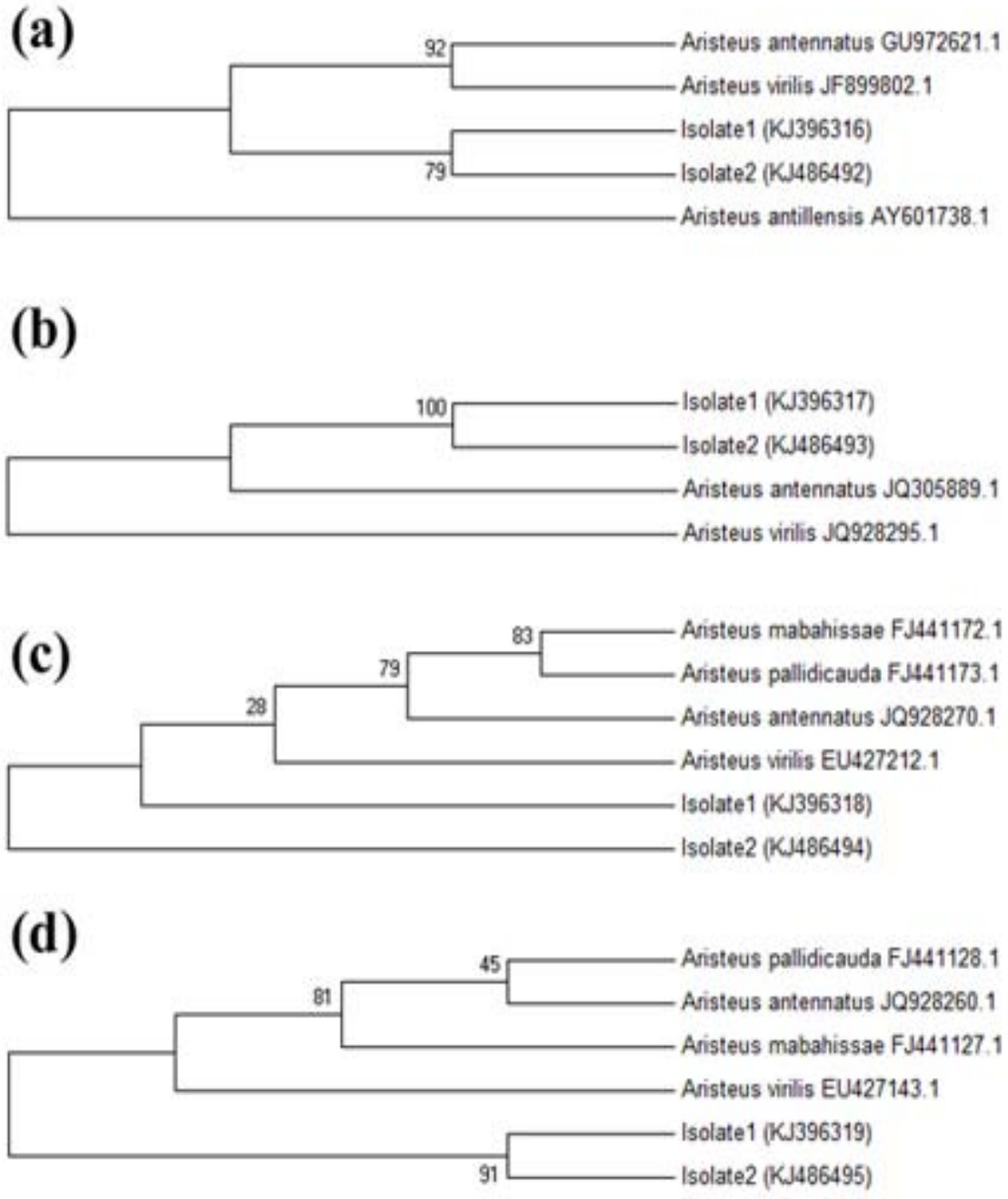

Fig. 6. Maximum parsimony tree based on the genetic markers (a) 16S rDNA, (b) COI, (c) PEPCK and (d) NaK showing the relationship of the specimen sample with other closely related species. Bootstrap support values of nodes indicating associations among species are shown.

Table 4. Pairwise genetic distance and sequence identity of the specimen sample with other closely related species. (-- denotes sequences not available in GenBank)

		Aristeus antennatus		*Aristeus virilise*		*Aristeus antillensis*	
		Genetic Distance	Sequence Identity (%)	Genetic Distance	Sequence Identity (%)	Genetic Distance	Sequence Identity (%)
16S rDNA	Isolate1 (KJ396316)	0.044	96	0.055	95	0.175	82
	Isolate2 (KJ486492)	0.044	97	0.055	95	0.175	81
COI	Isolate1 (KJ396317)	0.142	86	0.439	88	--	--
	Isolate2 (KJ486493)	0.142	85	0.439	88	--	--
PEPCK	Isolate1 (KJ396318)	0.004	99	0	99	--	--
	Isolate2 (KJ486494)	0.004	99	0	100	--	--
NaK	Isolate1 (KJ396319)	0.035	96	0.008	99	--	--
	Isolate2 (KJ486495)	0.033	96	0.006	99	--	--

The DNA sequences of each gene fragments from two isolates of the specimen are available in the GenBank.

The nucleotide sequences of all four gene fragments were subjected to pairwise genetic distance and phylogenetic analysis using MEGA5. Pairwise genetic distance and sequence identity of all genes with that of closely related species are given in the Table 4. There are several methods to assess phylogenetic relationships among species by using their nucleotide sequence variations of specific regions in the genome. The single gene phylogenetic tree constructed using the statistical method of maximum parsimony implemented in MEGA5 is shown in Fig. 6. This is the first report of DNA sequence submission of 16S rDNA, COI, PEPCK and NaK for *A. alcocki* in GenBank. Analysis of sequence identity, genetic distance and phylogenetic tree shows that the specimen has close similarity with *Aristeus antennatus*. But based on the morphological examination it was found that there are some minor morphological differences existing between *A. alcocki* and *A. Antennatus*. In *A. alcocki* 3rd abdominal tergum is rounded and sometimes ends with a minute spine, buttress of pterygostomian spine is well developed and curved anteriorly, length ratio chela/carpus of 1st periopod is 1.14 to 1.35, epipods are present from 1st to 4th pereiopods, number of photophores on propodus and carpus of 5th pereiopod are 28-34 & 21-26, respectively.

While in case of *A. antennatus* posterior end of 3rd abdominal tergum always ends with spine, buttress of pterygostomian spine is short and well formed, length ratio chela/carpus of 1st periopod is 1.30 to 1.48, epipods are present from 1st to 3rd pereiopods, number of photophores on propodus and carpus of 5th pereiopod are 73-98 & 65-117, respectively. Therefore, this study confirms the specimen as *A. alcocki* and the DNA sequence data available in the GenBank will be a contribution towards identification of *A. alcocki* on molecular basis in future.

Conclusion

This study has identified the specimen as *A. alcocki* on the basis of morphometric examination as well as molecular characterization of phylogenetic genes, and therefore reports the occurrence of *A. alcocki* in trawls off Sakthikulangara from southwest coast of India.

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References

1. Nezhad S R K, Modheji E & Zolgharnein H, Polymorphism analysis of mitochondrial DNA control region of hawksbill turtles (*Eretmochelys Imbricata*) in ther Persian Gulf. *Indian J Mar Sci,* 42 (2013) 300-303.
2. Linda K P & Paul M, Developments in molecular genetic techniques in fisheries, in: *Molecular Genetics in Fisheries*, edited by Carvalho GR & Pitcher TJ, (Chapman and hall, London) 1995, pp. 1-28.
3. Avise J C, *Molecular Markers, Natural History and Evolution,* (Chapman and Hall, New York) 1994.
4. Asensio L, Gonzalez I, Fernandez A, Rodriguez M A, Lobo E, Hernandez P E, Garcia T & Martin R, Application of random amplified polymorphic DNA (RAPD) analysis for identification of grouper (*Epinephelus guaza*), wreckfish (*Polyprion americanus*), and nile perch (*Lates niloticus*) fillets. *Journal of Food Product,* 65 (2002) 432-435.
5. Backer J, Bentzen P & Moran P, Molecular markers distinguish coastal cutthroat trout from coastal rainbow trout/steelhead and their hybrids. *Transaction of American Fisheries Society,* 131 (2002) 404-417.
6. Rasmussen C, Ostberg C O, Clifton D R, Holloway J L & Rodriguez R J, Identification of a genetic marker that discriminates ocean-type and stream-type Chinook salmon in the Columbia River Basin. *Transaction American Fisheries Society,* 132 (2003) 131-142.
7. Ganopoulos I, Aravanopoulos F, Madesis P, Pasentsis K, Bosmali I, Ouzounis C & Tsaftaris A, Taxonomic Identification of Mediterranean Pines and Their Hybrids Based on the High Resolution Melting (HRM) and trnL Approaches: From Cytoplasmic Inheritance to Timber Tracing. *PLoS One,* 8 (2013).
8. Shekhar, M S & Chandra P K, Restriction profile of amplified mitochondrial DNA of wild shrimp (*Penaeus monodon* Fabricius) populations of India. *Indian J Mar Sci,* 29 (2000) 65-68.
9. Stillman J H & Reeb C, Molecular phylogeny of eastern Pacific porcelain crabs, genera Petrolisthes and Pachycheles, based on the mtDNA 16s rDNA sequence: Phylogeographic and systematic implications. *Mol Phylogenet Evol,* 19 (2001) 236-245.
10. Birky C W, Fuerst P & Maruyama T, Organelle gene diversity under migration, mutation, and drift: equilibrium expectations, approach to equilibrium, effect of heteroplasmic cells, and comparison to nuclear genes. *Genetics,* 121 (1989) 613-627.
11. Tsang L M, Ma K Y, Ahyong S T, Chan T Y & Chu K H, Phylogeny of Decapoda using two nuclear protein-coding genes: origin and evolution of the Reptantia. *Mol Phylogenet Evol,* 48 (2008) 359–368.
12. Leys R, Cooper S J B & Schwarz M P, Molecular phylogeny and historical biogeography of the large carpenter bees, genus Xylocopa (Hymenoptera: Apidae). *Biol J Linn Soc Lond,* 77 (2002) 249–266.
13. Friedlander T P, Regier J C, Mittere C & Wagner D L, A nuclear gene for higher level phylogenetics: phosphoenolpyruvate carboxykinase tracks mesozoic-age divergences within Lepidoptera (Insecta). *Mol Biol Evol,* 13 (1996) 594–604.
14. Alcock A, *A Descriptive Catalogue of the 'Indian Deep-sea Crustacea Decapoda, Macrura and Anomala, in the Indian Museum: Being a revised account of the deep-sea species collected by the Royal Indian Marine Survey Ship "Investigator",* (Trustees of the Indian Museum, Calcutta) 1901.
15. Silas E G, Exploratory fishing by R.V. Varuna *Bull Cent Mar Fish Res Inst,* 12 (1969) 1-86.
16. Mohamed K H & Suseelan C, *Deepsea prawn resources off the southwest coast of India. Abstracts, Symp Living Resources of the Seas around India*, (CMFRI, India) 1968, pp. 27.
17. Suseelan C, Taxonomic notes on a potentially commercial deep-sea prawn from the southwest coast of india. *J Mar Biol Ass India,* 31 (1989) 54-58.
18. Ramadan M M, Crustacea, Penaeidae. *Scientific Reports of the John Murray Expedition,* 5 (1938) 35-76.
19. Kensley B, Tranter HA & Griffin D J G, Deepwater decapod Crustacea from eastern Australia (Penaeidea and Caridea). *Records of the Australian Museum,* 39 (1987) 263-331.
20. Crosnier A, Crustaces Decapodes Peneides Aristeidae (Benthesicyminae, Aristeinae,Solenocerinae). *Faune de Madagascar,* 46 (1978) 1-197.
21. Defreitas Antonio J, The Penaeodea of southeast Africa: II. The Families *Aristeidae* and *solenoceridae. Invest Rep Oceanogr Res Inst,* 57 (1985) 1-69.
22. Palumbi S R, Nucleic acids, II: the polymerase chain reaction, in: *Molecular Systematics,* edited by Hillis D M, Moritz C & Mable B K, (Sinauer Associates, Inc., Sunderland, Mass) 1996.
23. Folmer O, Black M, Hoeh W, Lutz R & Vrijenhoek R, DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Mol Mar Biol and Biotechnol,* 3 (1994) 294-299.
24. Sanger F, Nicklen S & Coulson A R, DNA sequencing with chain-terminating inhibitors. *Proc Natl Acad Sci USA,* 74 (1977) 5463-5467.
25. Thompson J D, Gibson T J, Plewniak F, Jeanmongon F & Higgins D G, The CLUSTAL X windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res,* 25 (1997) 4876-4882.
26. Nei M & Kumar S, *Molecular evolution and phylogenetics,* (Oxford University Press, Oxford) 2000.
27. Le Cren C D, The length-weight relationship and seasonal cycle in gonad weight and condition in perch, Perca fluviatilis. *J Anim Ecol,* 20 (1951) 201-209.
28. Petrakis G & Stergiou K I, Weightlength relationships for 33 fish species in Greek waters. *Fish Res,* 21 (1995) 465-469.

29. King M G & Moffitt R B, The sexuality of tropical deepwater shrimps (Decapoda; Pandalidae). *J Crustacean Biol,* 4 (1984) 567-571.
30. Kunda M, Dewan S, Uddin M J, Karim M, Kabir S & Uddin MS, Length-Weight relationship, condition factor and relative condition factor of *Macrobrachium rosenbergii* in rice fields. *Asian Fish Sc,* 21 (2008) 451-456.
31. Primavera J H, Parado-Estepa F D & Lebata J L, Morphometric relationship of length and weight of giant tiger prawn *Penaeus monodon* according to life stage, sex and source. *Aquaculture,* 164 (1998) 592-601
32. Kensley B, The south African Meseum's Meiring Naude Cruises part 5: Crustacea, Decapoda, Reptantia and Natantia. *Ann S Afr Mus,* 74 (1977) 13-44.
33. George M J, *On a collection of penaeid prawns from the offshore waters of the southwest coast of India. Proc Symposium on Crustacea*, (Marine Biological Association of India) 1966, pp. 337-346.