Available online at: www.mbai.org.in doi: 10.6024/jmbai.2016.58.1.1865-13

DNA barcoding of fiddler crabs *Uca annulipes* and *U. perplexa* (Arthropoda, Ocypodidae) from the southwest coast of India

K. P. Apreshgi, K. V. Dhaneesh, Tresa Radhakrishnan and A. Biju Kumar*
Department of Aquatic Biology and Fisheries, University of Kerala, Thiruvananthapuram-695 581, Kerala, India.

*Correspondence e-mail: abiju@rediffmail.com

Received: 28 Jul 2015, Accepted: 30 May 2016, Published: 10 Jun 2016

Short Communication

Abstract

DNA barcoding of fiddler crabs *Uca annulipes* and *U. perplexa* from the mangrove ecosystems of the southwest coast of India has been carried out. The sequencing of mitochondrial CO1 gene confirmed the identification of the species. The record of the specimen *U. perplexa* from the eastern Arabian Sea shows the continuous distribution of the species from African coast to the Bay of Bengal.

Keywords*: fiddler crabs, DNA barcoding, Kerala, distribution.*

Introduction

Fiddler crabs (Crustacea: Ocypodidae) of the genus *Uca* Leach, 1814 are the most conspicuous macrobenthic faunal components in the tropical and subtropical mudflats and in the mangrove ecosystems (Crane, 1975). These crabs are intertidal and live in proximity to the mangroves or other coastal vegetation and have a significant role in detritus formation, nutrient recycling and dynamics of the ecosystem, along with other organisms living in the sediment (Bandekar *et al.*, 2011). The diversity of fiddler crabs is represented by over 100 species (Crane, 1975; Shih, 2012). Krishnan (1992) reported the distribution of fiddler crabs in India, and validated the presence of nine species in the east and west coasts of India and Andaman and Nicobar Islands and recorded such as *Uca lactea, U. dussumieri, U. urvillei* and *U. vocans* from the southwest coast of India. Pillay and Nair (1971) and Bandekar *et al.* (2011) recorded *U. annulipes* from the southwest coast of India. However, there is no published information on the molecular taxonomy of the fiddler crabs of Indian coast. This paper provides sequence data of the mitochondrial gene cytochrome oxidase 1 (CO1) of two fiddler crabs, *U. annulipes* (H. Milne Edwards, 1837) and *U. perplexa* (H. Milne Edwards, 1837) and the first record of the later species from the southwest coast of India.

Material and methods

The fiddler crabs were collected from the intertidal areas of Kadalundi mangrove (11° 07'N; 75° 50' E) and mangrove region of Puthuvypu Fisheries Station (10°37' N; 76° 12' E), Kerala State, India by hand picking during November 2013. The live specimens were brought to the laboratory and morphometric measurements namely carapace length

(CL) and carapace width (CW) were recorded to the nearest millimetre using digital Vernier Calliper (Aerospace, China) and weighed in gram using weighing balance (Shimadzu, Japan). The specimens were identified and classified using standard keys (Crane, 1975). Four specimens (two males and two females) used in the study are deposited in the museum collections of the Department of Aquatic Biology and Fisheries, University of Kerala, India (Voucher numbers DABFUK-AR-BR-52-55). Among them, specimens (n=2) were preserved in 95% ethanol for molecular taxonomy.

Total DNA was extracted from the chelate legs of the crabs using DNeasy Blood and Tissue Kit (QIAGEN, Hilden, Germany). Mitochondrial gene cytochrome c oxidase 1 (CO1) was amplified using the universal primers (LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer *et al.*, 1994) in a 25 μl reaction volume with *Taq* PCR master mix (QIAGEN, Hilden, Germany) using the thermal cycler (Eppendorf, Hamburg, Germany). Polymerase chain reaction products were purified for sequencing with USB ExoSAP-IT (Affymetrix Inc., Santa Clara, USA) and sequenced in forward and reverse direction with the PCR primers by Dideoxy Sanger standard method with BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems Inc., Foster City, USA) on an ABI sequencer (Applied Biosystems Inc., Foster City, USA). The resulting sequences were edited and aligned with BioEdit v.7.0.9.0. (IbisBiosciences, Carlsbad, USA., Hall, 1999). Species comparison of the candidate sequence to the most similar sequences was carried out with the available one from GenBank (http://www.ncbi.nlm.nih.gov/genbank/). Phylogenetic analysis and sequence divergence were estimated using the Kimura 2-Parameter distance model of MEGA (Version 6.0) Package (www.megasoftware.net/, Tamura *et al.*, 2013). Maximum likelihood tree was constructed and were bootstrapped 1,000 times to provide percentage bootstrap values for branch points. Genetic distance of each species was done based on pair-wise distance analysis using Maximum Composite Likelihood method (MEGA 6, Tamura *et al.*, 2013). Sequences were submitted in sequin format and are available in GenBank (KJ535695 and KJ535696).

Results and discussion

Systematic account

Class : Malacostraca
Order : Decapoda
Infraorder : Brachyura
Family : Ocypodidae Rafinesque, 1815
Subfamily : Ucinae Dana, 1851
Genus : *Uca* Leach, 1814

Uca annulipes

The carapace width and carapace length of males (n=2) varied between 10-17 mm and 6-11 mm respectively, whereas in the case of females (n=4) it varied between 7-13 mm and 3-7 mm respectively which were collected from mangrove regions of Kadalundi and Puthuvypu Fisheries Station, Kerala, (Voucher numbers DABFUK-AR-BR-52, 53).

Remarks: *U. annulipes* is distributed in India (Puducherry (Crane, 1975), Mumbai (Altevogt, 1955), Karwar (Bandekar *et al.*, 2011) and Cochin (Pillay and Nair, 1971), Madagascar (Hoffmann, 1874), Mozambique (Fourmanoir, 1953), Red Sea (Laurie, 1915), Republic of Mauritius (Crane, 1975), Seychelles (Borradaile, 1907) and South Africa (Stebbing, 1917).

Uca perplexa: In *U. perplexa*, the carapace width varied between 8-13 mm and carapace length varied between 5-8 mm in males (n=2), whereas in females (n=3), the width and length of carapace ranged from 4-7 mm and 3-6 mm respectively, which were collected from mangrove regions of Kadalundi and Puthuvypu Fisheries Station, Kerala, India (Voucher numbers DABFUK-AR-BR-54, 55) (Figs. 1 a, b).

Description: Gonopod with strong torsion, posterior flange slightly longer than anterior and clearly wider, the pore being set in a rather broad and very shallow notch; both flanges relatively short and broad compared with those of *U. annulipes*. Gonopore with marginal lip only slightly tilted, scarcely projecting up into pore's cavity, much less than in *U. annulipes*; rim of lip corneous brown; axis of lip directed obliquely forward, not toward midline as in the other subspecies. Major cheliped: Outer pollex very rarely with an indistinct row of minute tubercles, discernible among a usually general distribution of tubercles, making the outer pollex distinctly rough in most individuals of this subspecies; supramarginal keel and groove absent; predistal tooth moderate to large except in areas of mingling with annulipes, where it is sometimes small. Dactyl arched only in about distal fifth, starting at beginning of the strong, downward curve; more proximally its upper margin is virtually straight and almost parallel to edge of gape; the dactyl's breadth beyond proximal end is therefore greater in most individuals than usual in other subspecies, and in many individuals the dactyl is widest slightly distal to the middle; gape correspondingly narrowed; accordingly, the dactyl's width is greater than that of adjacent part of gape; outer surface of dactyl always smooth and somewhat flatter than usual in other subspecies.

Remarks: New record of *U. perplexa* along the west coast of India confirms the extended distribution of it towards

Fig.1a. *Uca perplexa* (Male)

Fig. 1b. *Uca perplexa* (Female)

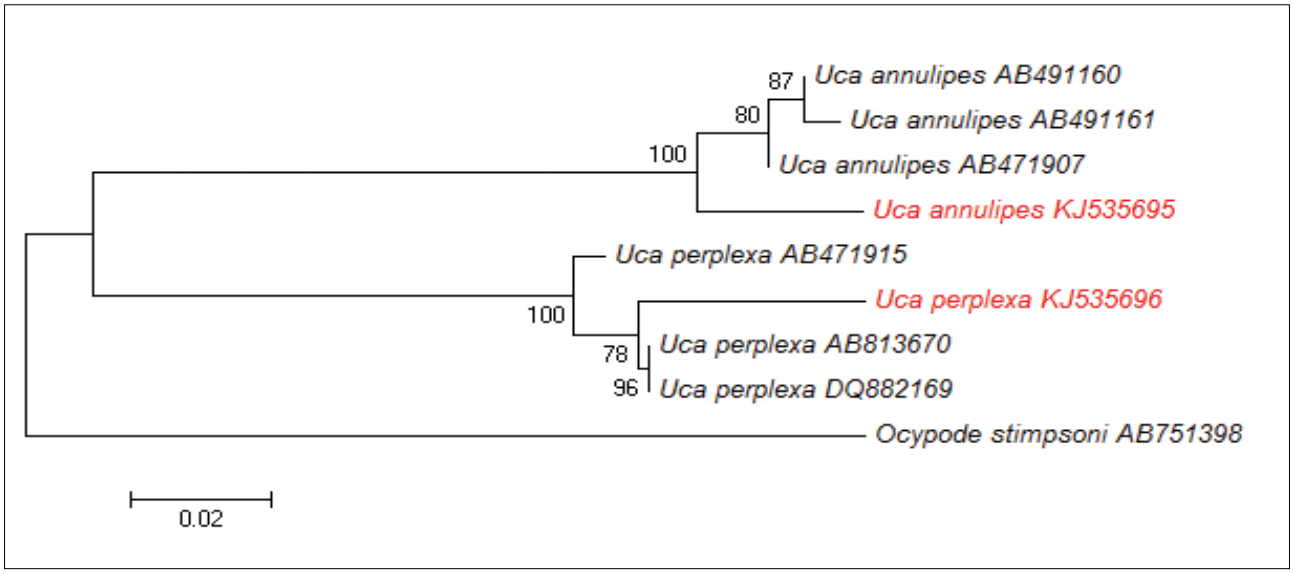

Fig. 2. Maximum Likelihood (ML) tree of Cytochrome oxidase subunit 1 (CO1) sequences of fiddler crabs from southwest coast of India along with the similar sequences

the south eastern Arabian Sea. Its wide spread distribution was also recorded from the East coast of India, Philippines, Singapore, Madagascar, east coast of Africa, Japan and Australia (Crane, 1975).

Phylogenetic analysis

The amplified sequences (average 648 bp) were larger than 600 bp, the limit typically observed for NUMTs (nuclear DNA sequences originating from mtDNA). The GC content of the sequences was 38.79% and 40.80% with a mean value of 39.8 ± 1.42%. GC-rich DNA is assumed to produce a more heat-stable helix and thus can be selectively advantageous in animals with high metabolic regulation induced by environmental drivers such as light, temperature, salinity, oxygen, and pH (da Silva *et al.*, 2011). Blast analysis showed 97% similarity with similar species available in the GenBank (AB471907 and AB813670), which reveals the possibility of the same species. According to maximum likelihood analysis, the sequence of *U. annulipes* and *U. perplexa* of the present study is well clustered to the clade of the similar species available in GenBank (Figure 2). Based on the sequence divergence, sequences of *U. annulipes* has genetic distance of 0.0393±0.005% and *U. perplexa* has 0.038±0.007% with its similar species

Table 1. Genetic distance of *U. annulipes* (KJ535695) and *U. perplexa* (KJ535696) with similar sequences from NCBI database.

Sl no	Species	1	2	3	4	5	6	7	8	9
1	*U. annulipes* KJ535695	0								
2	*U. annulipes* AB471907	0.0340	0							
3	*U. annulipes* AB491160	0.0393	0.0049	0						
4	*U. annulipes* AB491161	0.0446	0.0099	0.0049	0					
5	*U. perplexa* KJ535696	0.1917	0.1886	0.1887	0.1905	0				
6	*U. perplexa* AB813670	0.1934	0.1806	0.1807	0.1826	0.0339	0			
7	*U. perplexa* DQ882169	0.1934	0.1806	0.1807	0.1826	0.0339	0	0		
8	*U. perplexa* AB471915	0.1889	0.1762	0.1763	0.1781	0.0463	0.0149	0.0149	0	

(AB471907, AB491160, AB491161, AB813669, AB813670 and AB471915) (Table 1).

According to the maximum likelihood tree of fiddler crabs, barcodes of the same species get clustered in same clad, and it is clear that barcodes of the same species do not contain much variations. Sequences of *U. annulipes* were grouped in one main clad with the similar species and those of *U. perplexa* were grouped in another main clad with higher boot strap values.

Identification of species with similar appearance is challenging and DNA barcoding provide new perspectives in ecology and systematics of them. This study has strongly validated the efficacy of CO1 barcodes for identifying crab species. In this paper, we report the fiddler crab *U. perplexa* from the west coast of India for the first time and provide baseline details on the mitochondrial DNA sequences of *U. perplexa* and *U. annulipes* occurring in Indian waters.

Acknowledgements

The authors thank Dr. Hsi-Te Shih, Professor, Department of Life Science, National Chung Hsing University, Taiwan for confirming identification of species.

References

Altevogt, R. 1955. Some studies on two species of Indian fiddler crabs *Uca marionis nitidus* (Dana) and *U. annulipes* (Latr.). *J. Bombay Nat. Hist. Soc.,* 52: 702-716.

Bandekar, P. D., K. Neelkantan and V. S. Kakati. 2011. Biodiversity of crabs in Karwar mangrove environment west coast of India. *Recent Res. Sci. Technol.,* 3(4): 1-5.

Borradaile, L. A. 1907. Land and freshwater Decapoda (Percy Sladen trust expedition to the Indian Ocean in 1905). *Trans. Linn. Soc. Lond.,* 2 (12): 63-68.

Crane, J. 1975. Fiddler crabs of the world (Ocypodidae: genus Uca). Princeton University Press, New Jersey, 736pp.

da Silva, J. M., S. Creer, A. dos Santos, A. C. Costa, M. R. Cunha, F. O. Costa and G. R. Carvalho. 2011. Systematic and evolutionary insights derived from mtDNA COI barcode diversity in the Decapoda (Crustacea: Malacostraca). *PLoS ONE* 6 (5) e19449: 1-15.

Folmer, O., M. Black, W. Hoeh, R. Lutz and R. Vrijenhoek. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Mol. Mar. Biol. Biotech.,* 3: 294–299.

Fourmanoir, P. 1953. Notes sur la faune de la mangrove dans la région de Majunga: Crabs, Crebettes, Poissons. *Le Naturaliste Malgasche.,* 5(1): 87-92.

Hall, T. A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysisprogram for Windows 95/98/NT. *Nucleic Acids. Symp. Ser.,* 41: 95-98.

Hoffmann, C. K. 1874. CrustacésetEchinodermes de Madagascar et de l'île de la Réunion. In: Pollen, F.P.L. et D.C. van Dam, Recherchessur la faune de Madagascar et de sesdépendancesd'après les découvertes de François 5(2): 1-58 (referred in abstract).

Krishnan, S. 1992. Distribution of fiddlers in India. *Rec. Zool. Surv. India.* 91(3-4): 471-474.

Laurie, R. D. 1915. Reports on the marine biology of the Sudanese Red Sea, from collections made by Cyril Crossland, M.Z. on the Brachyura. *Zool. J. Linnean. Soc.*, 31(209): 407-475.

Pillay, K. K. and N. B. Nair. 1971. The annual reproductive cycles of *Uca annulipes, Portunus pelagicus and Metapenaeus affinis* (Decapoda: Crustacea) from the South-west coast of India. *Mar. Biol.,* 11: 152-166.

Shih, H. T. 2012. Distribution of fiddler crabs in East Asia, with a note on the effect of the Kuroshio Current. *Kuroshio Science,* 6(1): 83-89.

Stebbing, T. R. R. 1917. The Malacostraca of Natal. I. *Annals of Durban Museum,* 2(1): 1-33.

Tamura, K., G. Stecher, D. Peterson and S. Kumar. 2013. MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. *Mol. Biol. Evol.,* 30: 2725-2729.