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A Genetical and Ecological Diversity of Fresh Water Prawns *Macrobrachium Canarae* and *Caridina Gracilirostris* from Kanyakumari Dist., Tamil Nadu, India

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Abstract

Fresh water prawns are cultured widely around the world but little is known about the levels and patterns of genetic diversity. This paper reports the RAPD analysis of two species of fresh water prawns of Atydiae and Palaemonidiae family and the genus *Macrobrachium* and *Caridina* collected from Kanyakumari district. Specimens are identified using species-specific morphological characteristics. Morphological characters have limitations of describing intra specific genetic diversity as they are polygenic and expressions can be modified by the environment. The advent of molecular technique made possible not only the genetic analysis and also the study of evolutionary relationship. Molecular analysis of the morphologically identified species are done by extracting the DNA from the animal and concentration of DNA is measured using Nanodrop spectrophotometer at a wavelength of 280nm. Further analysis was performed with RAPD (Rapid Amplified Polymorphic DNA) a PCR (Polymerase Chain Reaction) based technique. It consist of genomic DNA, amplified with randomly constructed oligonucleotides. Specific quantity of extracted DNA was then amplified by multiplex PCR using random primers and 16S rRNA gene products was then analyzed using a bioanalyzer. From the analysis, electropherograms of DNA samples were obtained and the electrophoresis gel image of the samples were obtained. Electropherogram were then overlaid on each other and the genetic variation between the two species was determined.

Keywords: Electropherogram, RAPD, PCR, Bioanalyzer, Nanodrop spectrophotometer, Atydiae, Palaemonidiae, *Macrobrachium* and *Caridina.*

Introduction

The freshwater prawn is cultured widely around the world but little is known about the levels and patterns of genetic diversity. This rapid development and consequent land use is now threatening in inland freshwater bodies either causing degradation or destruction of ponds. The fresh water bodies mentioned in earlier literature are present now(Henderson, 1893, Kemp, 1915, Richard, 1983, Mariappan and Richard, 2006) in the urban surroundings. The fresh water fauna in general, the fresh water prawns in particular face the danger of being swept away from the environment. Thus a dire necessity to record, study and save the natural fauna of fresh water prawns in and around the urban areas exists. A proper survey and molecular studies of, these neglected forms of an essential prerequisite for any such study.

Realizing the need for the survey and identification of these highly distributed prawns from natural ecosystem, as a prerequisites for any biological research and culture prospects, noteworthy contributions have been made from several parts of the world. Mention may be made mainly of the following very recent works from the Indian subcontinent, Natarajan (1942), Chopra and Tiwari (1947), Tiwari (1949, 1952, 1958, 1964). Tiwari and Pillai (1968, 1971, 1973), Pillai (1964), Chinnayya (1971), Jalihal and Sankolli (1975a, 1975b, 1979a, 1979b), Anantharaman *et al* (1978) Ravindranath (1977, 1979, 1981), Jalial (1978), Jalial *et al* (1981, 1983, 1984, 1994), Shenoy *et al* (1984, 1993), Jayachandran and Joseph (1985, 1986) and Chandra (1994).

Mitochondrial DNA sequence were used to investigate phylogenetic and biogeography relationship among Australian fresh water shrimps from the genus *Caridina* H.Milne Edward, 1937 (Atyidae) and cingeners from potential source populations throughout the west Indo-west pacific region Page T.J, *et al*, 2005.

Multiple origin of endemic Australian *Macrobrachium* based on phylogenetic relationship among the seven endemic and six non endemic Australian *Macrobrachium* along with five non- Australian species, were referred from the mitochondrial 16S rRNA gene sequence (Murphy, Austin 2004).

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However these studies covered a very small geographical area and included the Atyid prawns only. The scanning of literature revealed the paucity of information of the taxonomy and distribution of freshwater spread of this district and on its suburban position, surrounding the fast developing metropolis of Chennai.

Kanyakumari district, Tamil Nadu, India is at the tip of the Indian peninsula and faces the Indian Ocean. The district is generally hilly with plains found near the coast, the land from the sea-coast gradually hilly, with plains found near the coast. The land from the sea coast gradually rises from sea level to the Western-Ghats hills in the deep interior of the district. The district has 62 km of the coast on the western side (Arabian sea Coast) and 6 km of the coast of eastern side (Gulf of manner/ Bay of Bengal coast). It is the smallest district in Tamil Nadu with a land spread of 1,684 sq.km and has almost all ecosystems- forests, wet lands, fresh water resources,

marine, etc. The District, once called the “The Granary of Travancore” is fertile, with hundreds of water bodies and an excellent canal irrigation system. Rubber and spice plantations are found on the hilly terrain, while paddy fields, plantain (banana) and coconut plantation are found on the plain, near the coast.

The material for study was collected from the different sites in and around the Kanyakumari district covering rivers, lakes, ponds and brackish water systems of this district.

Materials and methods

Morphological identification

The specimens are examined under compound microscope to identify the species using species - specific morphological characteristics

The various organs that are examined for identification include:

1. Rostrum - anterior part of carapace
2. Antennule
3. Antenna
4. Mandible
5. First maxilla
6. Second maxilla
7. First maxilliped
8. Second maxilliped
9. Third maxilliped

Morphological characters have limitations of describing intraspecific genetic diversity as they are polygeneic and expression can be modified by the environment. Their use in population stock identification studies has been superseded for the most part by the development of district genetic methods. Morphological studies of the animal was done and the species of the animal was identified.

Molecular Analysis

The DNA was extracted from the animal by standardized procedure and the concentration of the DNA measured using Nanodrop spectrophotometer at a wave length of 280nm. The nanodrop is a cuvette free spectrophotometer. It uses just 1 micro liter of measure from 5ng/μl to 3000ng/μl. The nanodrop has greatly improved our sequencing success rates and our ability to troubleshoot problems more effectively.

Further analysis was performed with RAPD (Rapid Amplified Polymorphic DNA) a PCR based technique (Polymerase Chain Reaction), developed by Williams *et al.* (1990). Welsh and McClelland (1990), Caetano Anolles (1991). It consist of genomic DNA, amplified with randomly constructed oligonucleotides. Specific quantity of the extracted DNA was then amplified by Multiplex PCR using random primers and the 16S rRNA gene product is obtained.

Primers

1. 5’-GGT-GCG-GGA-A-3’
2. 5’-GTT -TCG-CTC-G -3’
3. 5’ -GTA-GAC-CCG-T-3’
4. 5’ -CAG-AGC-CCG-T-3’
5. 5’ -AAC-GCG-CAA-C-3’
6. 5’ -ACC-GTC-AGC-A-3’

Electrophoresis studies using bioanalyzer

The amplified product was then analyzed using a bioanalyser. A agilent 2100 Bioanalyzer is a personal Lab-on-a Chip platform operating disposable micro fluidic chips for analysis of RNA/DNA /Protein and cells. In the bioanalyser the samples are added in the wells on a chip and the DNA fragments are separated by molecular sieving and analyzed inside the chip, which uses electrode for separating the DNA fragments and uses a light source to detect the DNA fragments. From the analysis, electropherograms of the DNA samples of the four species were obtained and the electrophoresis gel image of the samples were obtained. A standard ladder of size range from 15 to 1500bp was used. The electropherograms were then overlaid on each other and the genetic variation between the four species was determined

Results

Morphological analysis

The animal collected from different parts in the district of Kanyakumari were morphologically identified as

Macrobrachium canarae (Tiwari, 1958)

Caridina gracilirostris (de Man, 1892)

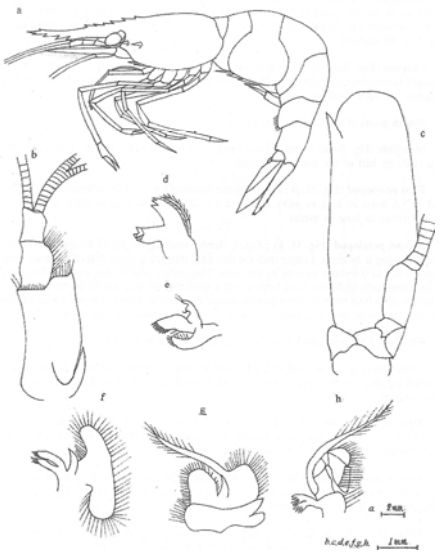


Figure 1: *M. canarae.*

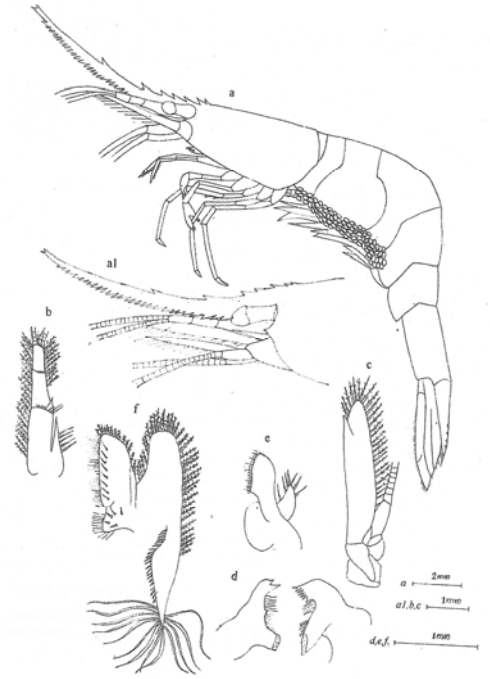


Figure 2: *C. gracilirostris.*

Description

Macrobrachium canarae

Rostrum (Fig 1 a): straight, longer than the antennules peduncle and antennal scale.
Rostral formula $\frac{6 - 9}{4 - 6}$ usually $\frac{6 - 8}{4 - 5}$ with 1 or 2 postorbital teeth.

Antennule (Fig 1 b)
Antenna (Fig 1 c)
Mouth parts (Fig 1 d to h)
Mandible (Fig d)

Caridina gracilirostris

Rostrum (Fig 2a & 2a1): Distinctly out reaching the antennal scale and the antennuler peduncle, distal end of the rostrum upturned,
Rostral formula $\frac{7 - 8}{27 - 37}$ mostly $\frac{7 - 8}{34 - 36}$ with 1 or 2 postorbitals.

Carapace (Fig 2 b)
Antennule (Fig 2 b)
Antenna (Fig 2 c)
Mouth parts (Fig 2 d,e,f)
Mandible (Fig 2 d)

Molecular analysis

The DNA extracted from the tissue sample were analyzed using a bioanalyzer. The

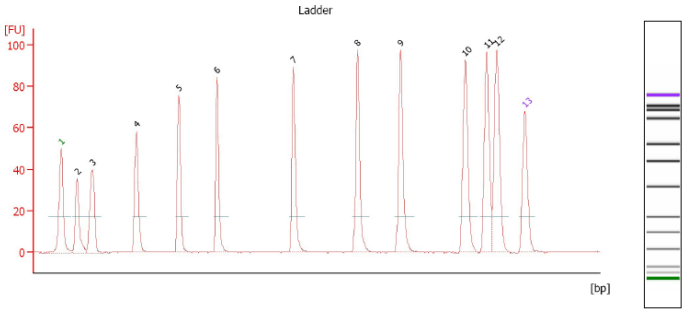
result of the analysis are given. The results of the RAPD analysis can be deciphered from the electropherogram given below.

From the electropherogram results of *Macrobrachium canarae and Caradina gracilirostris.*

The peaks obtained were 6 for *Macrobrachium canarae.* The peaks were obtained in base pairs 15, 35, 129, 156, 216, 478, 518, 1,500 with the concentration of 4.20, 2.33, 0.50, 0.47, 0.37, 0.43, 0.87, 2.10 (ng/µl) respectively.

The peaks obtained were 4 for *Caradina gracilirostris.* The peaks were obtained in base pairs 15, 23, 184, 273, 293, 1500 with the concentration of 4.20, 3.22, 0.96, 0.85, 1.11, 2.10 (ng/µl) respectively.

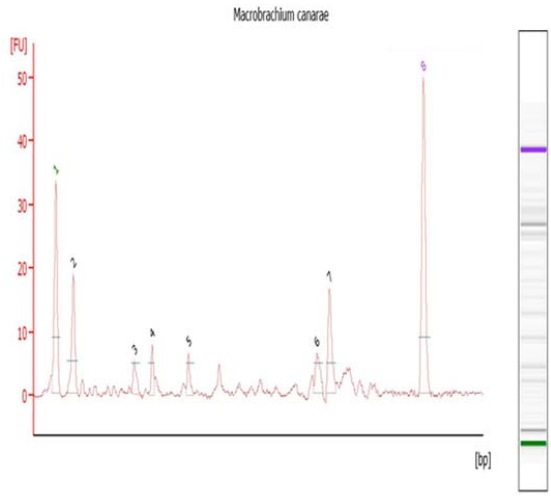
The DNA sample of all the two species were analyzed. A standard ladder of size range from 15-1500 base pairs was used.



Peak table for Ladder

Peak	Size [bp]	Conc. [ng/µl]	Molarity [nmol/l]	Observations
1	15	4.20	424.2	Lower Marker
2	25	4.00	242.4	Ladder Peak
3	50	4.00	121.2	Ladder Peak
4	100	4.00	60.6	Ladder Peak
5	150	4.00	40.4	Ladder Peak
6	200	4.00	30.3	Ladder Peak
7	300	4.00	20.2	Ladder Peak
8	400	4.00	15.2	Ladder Peak
9	500	4.00	12.1	Ladder Peak
10	700	4.00	8.7	Ladder Peak
11	850	4.00	7.1	Ladder Peak
12	1,000	4.00	6.1	Ladder Peak
13	1,500	2.10	2.1	Upper Marker

Figure 3



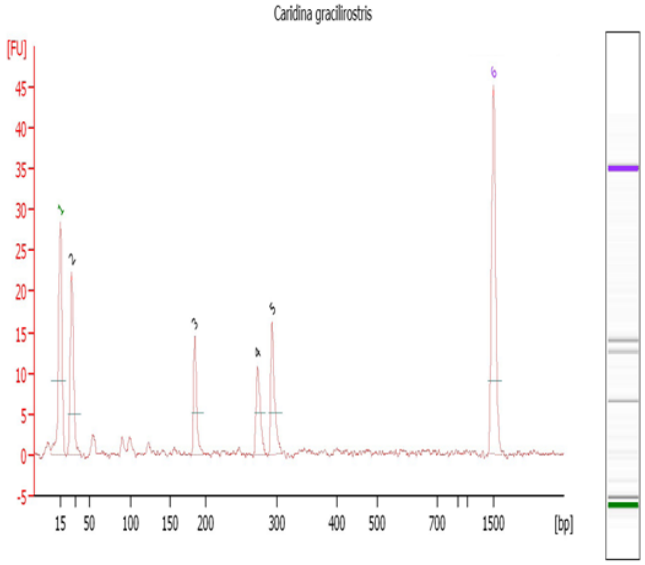
Overall Results for sample

Number of peaks found: 6

Peak table for sample

Peak	Size [bp]	Conc. [ng/µl]	Molarity [nmol/l]	Observations
1	15	4.20	424.2	Lower Marker
2	35	2.33	101.2	
3	129	0.50	5.9	
4	156	0.47	4.6	
5	216	0.37	2.6	
6	478	0.43	1.4	
7	518	0.87	2.5	
8	1,500	2.10	2.1	Upper Marker

Figure 4



Overall Results for sample

Number of peaks found: 4

Peak table for sample

Peak	Size [bp]	Conc. [ng/µl]	Molarity [nmol/l]	Observations
1	15	4.20	424.2	Lower Marker
2	23	3.22	215.2	
3	184	0.96	7.9	
4	273	0.85	4.7	
5	293	1.11	5.8	
6	1,500	2.10	2.1	Upper Marker

Figure 5

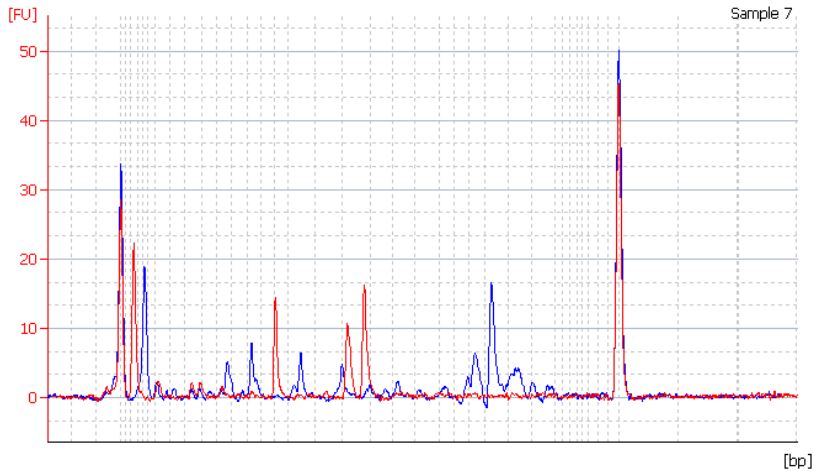


Figure 6

Overlapped Electropherogram image of *Caridina gracilirostris* and *Macrobrachium canarae*

The genetic difference between the two species could thus be observed from the peak variation in the electropherogram.

The species *Macrobrachium canarae* were distributed only in land areas and *Caridina gracilirostris* were distributed only near sea areas. So the genetic variations are seen for these two species based on their distribution.

Discussion

The advent of molecular technique made possible not only the genetic analysis and also the study of evolutionary relationship among the species or phylogeny. Although there are many ways to study phylogenetic relationships example amino acid sequence of protein, nucleotide sequence of nucleic acid and presence or absence of enzyme, it is now recognized that ribosomal RNA is important indices or phylogeny. Ribosomal RNA has the characteristics that are important in studying the evolutionary divergences. These universal characteristics have identical functions in all living organisms. This functional constancy makes rRNA ideal molecular chronometers to measure evolutionary changes. Because rRNA is a small molecule that cannot tolerate much structural changes and still retains its function, its sequences moderately well conserved or constant across phylogenetic lines. Consequently small differences in rRNA sequence can be used to determine evolutionary distance. Among the rRNAs, 16S is used most commonly as phylogenetic tool. Small size rRNA limits the amount size rRNA makes this molecule more difficult to experimentally analyze.

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