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Research Article

**MOLECULAR PHYLOGENETIC VARIATION OF  
RHYNOCORIS MARGINATUS PREDICATED ON  
RIBOSOMAL 16S GENE (HETEROPTERA : REDUVIIDAE)****Bharathi T and Baskar A**Department of Zoology, TDA College Kanniraja puram -623135, Tamil Nadu, India.  
Department of Zoology, S.B.K. College, Aruppukottai- 626101, Tamil Nadu, India.**Abstract:**

The Assassin bugs of the genus *Rhynocoris* are mostly predatory and biocontrol insect pest with currently close to 90 species described worldwide. In this present study we investigate the genetic variation of Ribosomal gene 16s of *Rhynocoris marginatus*. The results showed that the *Rhynocoris marginatus* insect gene nucleotide sequence and hydrophobic peaks explain the more and less hydrophilic residues were calculated. And multiple gene sequence and phylogenetic variation were observed. When compared to other sequences, differentiation in the nucleotide and amino acid sequence of *R. marginatus* were reported.

**Keywords:** *Rhynocoris marginatus*, mtDNA, phylogenetics, sequence, RNA.

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**INTRODUCTION:**

Eukaryotic ribosomal DNA (rDNA) has several properties and was found useful for studying genetic variability and divergence within and between species.(Fritz *et al.*,1994). Assassin bugs have different morphs, biotypes, and ecotypes with various colours and shapes which often mislead a museum entomologist in recognizing the morphs and ecotypes of a particular species. (Dunston P.Ambrose *et al.*, 2014). The assassin bugs of the *Rhynocoris* species are well known for their role in bio control potential of the insect pests, yet their molecular relationships have not been established at molecular level (Putshko VG *et al.*,1985, Maldonado J;1990, Baskar *et al.*,2014). The typical insect mitochondrial genome is a circular, double stranded DNA molecule of about 12-20kb in length that contain 37 genes, 13 protein coding genes, 22 transfer RNAs (tRNA) and two ribosomal RNAs (rRNA) (Wolstenholme D *et al.*,1992, Bore AL ;1999 and Yu Nie *et al.* ,2020). Mitochondrial DNA has various interesting properties such as abundance in animal tissue, small size relatively simple genomic structure fast rate of evolution and a straight forward mode of transmission with a low level of recombination (due to its maternal inheritance) This makes it a valuable tool for comparative genomic resolution. (Avisé *et al.*,1987, Mortz *et al.*,1987 and Arthur Kocher *et al.*,2014). In this investigation was undertaken based on available in mitochondrial sequences of *Rhynocoris marginatus* and we are amplified and sequenced the partial 16s ribosomal gene from the mtDNA.

**MATERIALS AND METHODS:****Collection of *Rhynocoris marginatus***

The adult insect samples representing *Rhynocoris marginatus* were collected from Ayyanar Kovil Tropical Rain forest bordering an agro ecosystem (altitude 389 MSL, latitude 76. 39°E and 10.45°N) near Rajapalayam, Virudhunagar District, Tamil Nadu, Southern India, during 2018-2019. Minimum 10 insect samples were collected for each species and all the insect specimens were stored in absolute ethanol. Selected samples (n=5) were processed for DNA extraction following complete removal of ethanol. Total mtDNA was extracted from thoracic muscle or leg muscle of individual of the *Rhynocoris marginatus* by phenol-chloroform method with minor modification as described by [10] by addition of 30µl of proteinase k (20mg/ml) and incubated for 16 hr at 52°C.

**Polymerase Chain Reaction, sequencing and analysis**

An approximate of 636bp DNA fragment of the 16s gene was amplified for each *Rhynocoris marginatus* by two universal 16s gene specific primers: 16saF (5'-CGCCTGTTTATCAAAAACAT-3') and 16sb(5'CTCCGGTTTGAACTCAGATCA-3') as reported previously. The PCR products were separated on 1.5% agarose gel and visualized by ethidium bromide staining. The PCR products were purified using the HiYield PCR/ Gel extraction nkit (RBC Biosciences, Taiwan) following the manufacturer's instructions. The purified amplicons were sequenced using the Big Dye Terminator Cycle sequencing ready reaction kit (Applied Biosystems Inc.,USA) in the ABIprism 3100 Genetic analyzer. The sequencing of COI amplicons of each species (n=5) was performed with the forward and reverse primer, and consensus sequence. Sequenced COI gene of *Rhynocoris marginatus* was assembled and analysed EXPASY translate.

**RESULTS AND DISCUSSION:**

The 16s gene of *R. marginatus* has been partially sequenced and. Nucleotide sequencing of *R. marginatus* 16s gene amplicon disclose an average size of 636 bp (Figure 4). The A+T percentage for the *R. marginatus* 16s gene is 62% and G+C percentage is 38%. The analysis into divulge the nucleotide frequencies of A-27%, T-35%, C-19% and G-19% (Table 1).Hydropathy plot of the *in-silico* translated amino acid sequence of the *R. marginatus* 16s gene protein designates more of hydrophilic residues (mean by the peaks) and less of hydrophobic residues.(figure 2)Molecular weight of the *R. Marginatus* 16s gene in 53911.82µ and Residus 1-636, the average residus weight-85.635.Histogram plot of the *in silico* translated amino acid sequence of the *R.marginatus* 16s gene indicates position from 1 to 636 like, Tiny residues and aliphatic, aromatic, non polar, polar residues and positive and negative residues. The phylogeny was framework predicated on the aligned 16s gene sequences and 18s and 28s is a shown figure(4)The evolutionary history was inferred using the Neighbour joining method. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree (Baskar *et al.*,2010.) The phylogeny was created rested on the aligned 16s gene sequences and the tree obtained with the sum branch length of 0.29726 And here compared with three mitochondrial genes like 16s,18s and 28s. The phylogenetic relationship revealed the existence of three clusters. The gene of the 16s formed one cluster and another 18s and 28s grouped forming other two clusters. The present study demonstrated the great effectiveness of mitochondrial 16s gene for inferring phylogenetic

relationships at *Rhynocoris marginatus* insect mitochondrial and ribosomal gene level. Here reported to the phylogenetic relationship between the mitochondrial genes 16s,18s and 28s gene. Dunston P.Ambrose and sivakumar et al 2014 were studied and reported into intragenic phylogenetic relationships between thirteen species of *Coranus Curtis* and Eisuke Hasegawa et al.,2006 were reported the phylogenetic analysis of the insect order Odonata,Mahendran et al., 2006 were reported into Bombycidae. And already reported in other insects such as, *Chironomus* (Diptera)species Guryev et al 2001 and Jon m Mallat 2003, Yingqi lui et al 2022 in *Sigicoris* ,Austin et al,2004, K.p. Arunkumar et al.,2006 in *Bombyx mori* and yogesh et al 2000 also reported in similar gene in various insect orders.

### CONCLUSION:

The results obtained not only have enriched our knowledge on biosystematics but have also supplemented multidisciplinary data. The results further reveals the utility of 16s DNA sequence in multiple and phylogenetic analysis. Mitochondrial genomics holds considerable promise for unravelling long forgotten patterns of genome evolution. In the near future, comparison of mitochondrial genomic systems may help us better understand numerous patterns of evolution, including those of genomes and organisms.

### Acknowledgement

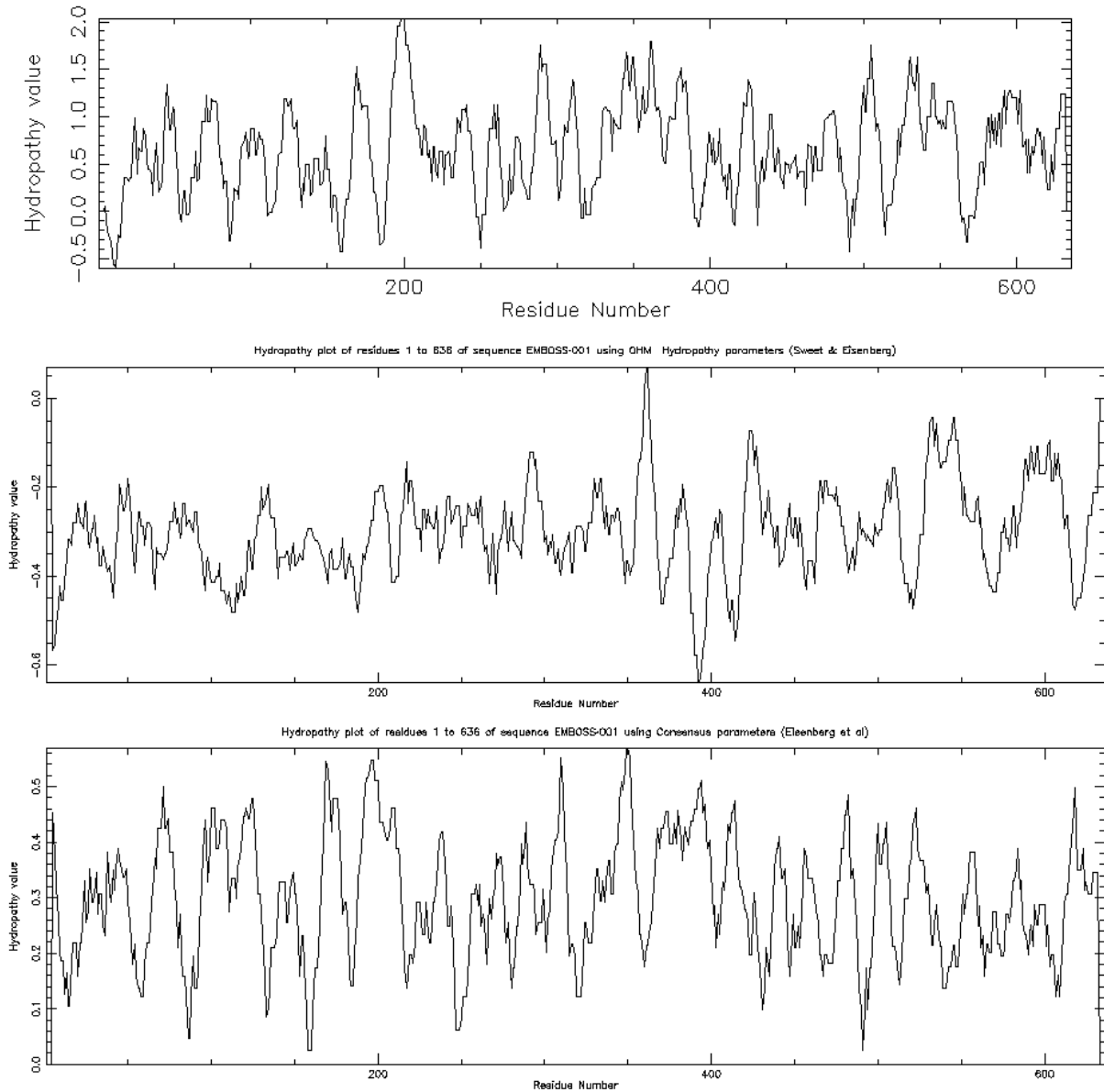
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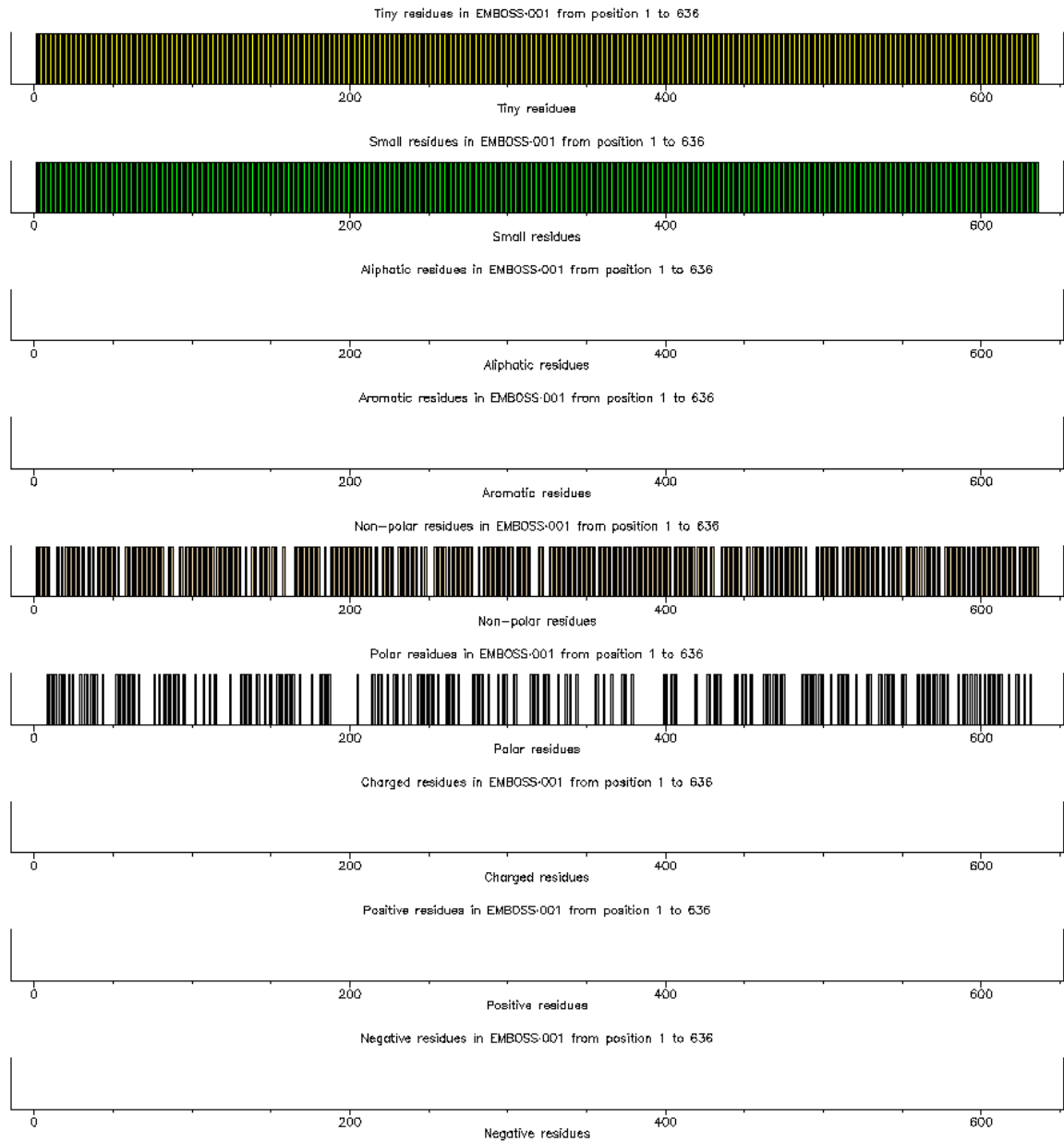
Hydropathy plot of residues 1 to 636 of sequence EMBOSS-001 using Kyte & Doolittle hydropathy paramet



**Figure1** Hydropathy plot of the in silico translated partial 16s gene protein from the 636 bp nucleotide sequence from *R.marginatus*.

**Table 1:** Nucleotide composition of the sequenced partial 16s gene from the *Rhynocoris marginatus*

No	Species name	Gene name	Nucleotide sequence obtained (bp)	A	A%	T	T%	C	C%	G	G%	AT%	GC%
1	<i>R.marginatus</i>	COI	636	173	27%	217	35%	122	19%	124	19%	62%	38%



## 5'3' Frame 1

Stop EVFWSYCALINIEWPRYFICAKAA Stop SFVF Stop LKAE Met NGNTKYKLSLNWLIIEFFLNKAEICL Stop NQKIL Stop Stop LTFAEFP  
 WIKFLFLQLSGGRHFIK Stop PSCAVEN Stop FLTF Stop PP Met IITKKTVSPGEYK Stop PRGGDII Met QGAVRPLIFHGAVI Stop LRGL  
 SCLSKRALFFN Met QYIFCGVENLPLTSLIF Stop HII Met CVIS Stop ACYPHTTLCCL Stop D Stop IDYI

## 5'3' Frame 2

G Stop C FGLTVHWL Met L N G P G I L S V L Stop QHNHLSFN Stop Stop LKW Met VGRSISFL Stop IG Stop LNFIF Stop Met K L K F V W G T K K P Stop G D L L  
 SLNFRSNSYFCYNYLVAGILLNPNFVPLKTDFWLLGLRWSLK Stop QFPLEN Met NNRAGGE Met S L C Stop GRFAP Stop PFTLSFNYG  
 AYPVCPSPGHYFL Met Y N I L S V E S Stop I S H L L S P L D T L L C V Stop F L E P A I L Met L L S V S K Met G L T I S

## 5'3' Frame 3

GGVLVLLCTD Stop YW Met A P V F Y L C Stop G S Met I I C L L I K G W N E W I D E V Q A F F K L A N W I L F F K Stop S W N L F E E P K N P K E T Y F R W I S V N Q I  
 L I F A T I I W P A F Y Stop Met I L Met C R W K Q I F D F Stop A S D D H Stop K D S S L P W Stop Met Stop Met I A R G G Stop Y H A G G G S P L N F S R S C H L I T G L I  
 L S V Q A G I I F Stop Y T Met H F L W S R E S P T Y F S H L Stop T H Y Y V C N F Stop S L L S S Y V S L S L Stop Stop D W L H H

## 3'5' Frame 1

V Met Stop S I L S Stop Stop Q Stop V V W G Stop Q A L E I T H Met Met C L K V Stop E V S G Stop P S T F Q K V Y C Met L K N N A R L D Stop Q D K F R N Stop Met T A P F  
 K I K G R T A P C Met Met Met S P P P R G Y L S P G E G T V F L V I I G G L K V N L F S T A H E G Y L Met K C R F P D N C S K N K N L I H G N S A K V S L L Stop V F W F  
 L K Q I S A L F K K Stop N S I S Q F K E S L Y F V Q P F I S A F N Stop K T N D Y A A L A Q Met K Y R G H S Met L I S A Q Stop D Q N T S

## 3'5' Frame 2

WCSQSYL Stop D Stop E Stop Y E D S Stop L Stop K L H T Stop Stop C V Stop Stop W E K Stop V G D S R L H Stop K C I V Y Stop K Met Met P A W I D Stop Met S P V I K W Q  
 L R E K L Stop G E P P P A Stop W Y L P P R A V I Y I L Q G Stop E L S F Stop W S S E A Stop K S K I C F Q R H Met Stop V I Stop Stop N A G H Q Met I V A K Met Stop I W F T  
 E I Q R K Stop V S L G F F G S S N K F Q L Y L K N K I Q L A N L K K A C T S S N H S F Q L I K Stop Q Met I Met L P Stop H Stop Stop N T G A I Q Y Stop S V H S K T K T P P

## 3'5' Frame 3

DVVNPILETESS Met Stop Met A G S Stop N Y T H N N V S K G E Stop S K W E I L D S I E S V L Y I K K Stop C P L G Q T G Stop A P Stop L N D S S V K N Stop G A N R P  
 L H N D I S P P A R L F Met F S Stop G N C L F S D H R Stop P K S Q K S V F N G T W L F N K Met F A T Stop Stop L Stop Q R Stop E F D S R K F S E S K S F Stop G F L V P  
 Q T N F S F I Stop K Met K F N Stop P I Stop Stop K L V L R P T I H F S L Stop L K D K W L C C L S T D K Met F G P F N I N Q C T V Stop P K H L P



Nucleotide sequence alignment of (16sgene)

## Phylogenetic Tree

*This is a Neighbour-joining tree without distance corrections.*

Branch length:  Cladogram  Real



18sgene 0.33969  
16s 0.23456  
28s 0.3093

## Tree Data

```
(  
18sgene:0.33969,  
16s:0.23456,  
28s:0.30930);
```

Phylogenetic Tree in relationship between (16s,18s and 28s).