Nucleotide Sequence and Phylogeny of *Rhynocorismarginatus* Fabricius based on 18S Ribosomal RNA Gene (Heteroptera: Reduviidae)

Abstract

The *R.marginatus* are essential components of ecosystem, but also important in the biological control of insect pest, infesting a varity of agro ecosystem and medicine. The present investigation was carried out in the insect molecular genetic variation of 18S Ribosomal RNA gene from *R. marginatus.* The study was represent by the reduviid insect*R. marginatus* nucleotide gene sequences were translate amino acid sequence and obtained hydropathy, Domain, Transmembranes of proteins were calculated. The multiple gene sequence alignment of in-silico translated amino acid sequence of the partial ribosomal genes protein of *R. marginatus* were generated and the phylogenetic relationships were observed.

Keywords

R. marginatus, Nucleotide Sequence, Phylogeney, 18S RNA, Genetic Variation

Introduction

Eukaryotic ribosomal DNA (rDNA) has several properties and was found useful for studying genetic variability and divergence within and between species.[1] The Assassin bugs of the genus *Rhynocoris* are from diverse group of mostly insect pest with currently close to 190 species described worldwide and it have different morphs, biotypes, and ecotypes with various colours and shapes and well known for their role in bio control potential of the insect pests, yet their molecular relationships have not been established at molecular level.[2,3,4,5]The typical insect mitochondrial genome is a circular, double stranded DNA molecule of about 12- 20 kb in length that contain 37 genes, 13 protein coding genes, 22 transfer RNAs (tRNA) and two ribosomal RNAs (rRNA).[6,7] 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.[8,9,10] In this investigation was carried out based on available in ribosomal gene sequences from *R.marginatus* and amplified the partial nucleotide sequence of 18S ribosomal RNA gene.

Materials and methods

Collection of *R. marginatus*

A laboratory colony of *R.marginatus*were collected from Ayyanar Kovil Tropical Rain Forest bordering an agro ecosystem (altitude 389 MSL, latitude 76. 39º E and 10.45º N) near Rajapalayam, Virudhunagar District, Tamil Nadu, Southern India, during 2018-2021. The adults emerged were allowed to mate and the *R. marginatus* reared in the laboratory were used for experimental studies. 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 *R. marginatus*by phenol-chloroform method with minor modification as described by addition of 30 µl of proteinase k (20 mg/ml) and incubated for 16 hrs at 52°C.

Polymerase Chain Reaction, Sequencing and Analysis

The PCR was carried out to amplify the partial 18S ribosomal genes of 826 bp DNA fragment amplify form *R.marginatus.* It was amplified using two universal 18S gene specific primers: 18sf (5'-AAATTACCCACTCCCGGCA-3') and 18sr (5' TGGTGUGGGTTTCCCGTGTT-3'). The PCR products were separated on 2% agarose gel and visualized by ethidium bromide staining. The PCR products were purified using the HiYield PCR/ Gel extraction kit (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 ABI prism 3100 Genetic analyzer. The sequencing of 18S amplicons from *R. marginatus* (n=5) was performed with the forward and reverse primer, and consensus sequence. Sequenced 18S gene of *R. marginatus* was assembled and analysedEditseq translate.

Results

We report here the isolation of the partial 18S ribosomal RNA gene sequence of the assassin bugs of *R. marginatus*. The 826 bp nucleotide sequence and conceptually translated aminoacid sequence of PCR amplicon of the 18S ribosomal RNA gene from *R. marginatus* (Figure 1). The nucleotide composition of A+T percentage for the *R. marginatus*18S gene is 53% and G+C percentage is 47%. The analysis into divulge the nucleotide frequencies of A-25%, T-28%, C-21% and G-26% (Table 1). In hydropathy plot of the in-silicotranslated partial 18S gene protein of the 826 bp nucleotide sequence from *R. marginatus.* 18S gene protein was designates more of hydrophilic residues (mean by the peaks) and less of hydrophobic residues (Figure 2).

Molecular weight of the *R. marginatus*ribosomal 18S gene in 67641.30µ and Residues 1-826, the average residues weight-81.890. Histogram plot of the in-silico translated nucleotide sequence of 18S gene indicates position from 1 to 826 bp, it refect tiny residues and aliphatic, aromatic, non polar, polar

residues and positive and negative residues of gene protein (Figure 3).

In-silico translation with invertebrate mitochondrial genetic code in the Editseq translate revealed of 257 amino acid sequences for *R. marginatus.* The translation of the partial nucleotide sequence and its deduced amino acid sequences are shown in figure 1. Because of the codon preference, the A+T composition in *R. marginatus* is particularly biased at the second codon position, which totaled 16.57%. The A+T content at first and third positions are 16.09% and 15.85% respectively. The G+C composition at first and third are 15.72% and 15.37% respectively (Table 1). Multiple sequence alignment was carried out in the partial nucleotide sequence of ribosomal genes 16S, 18S, 28S from *R. marginatus*. And it used to help for investigation of codon similarity and divergence.

Genetic distances between the examined *R. marginatus* in the 18S gene have been generated by Neighbor-joining method. The minimum value of genetic distance among the examined 18S gene sequence from *R. marginatus* was 3.64 when compared with 16S, 18S respectively (Table 3). It was also observed that the percentage identity of 28S with 18S was 48.75% with a divergence of 2.64% (Table 3). The phylogeny was framework predicated on the aligned 18S gene sequences and 16S 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.

The phylogeny was created rested on the aligned 18s gene sequences and the tree obtained with the sum branch length of 0.33279. And here compined with three Ribosomal genes like 18S, 16S 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 18S gene for inferring phylogenetic relationships at *R.marginatus* insect ribosomal gene level. Here reported to the phylogenetic relationship between the Ribosomal genes 16S, 18S and 28SRNA gene. (Figure 4)

act cta ttg agg ccc cgt aat cgg aat aga gta cac ttt aaa tcc ttt aac aag gat cca 60

T L L R P R N R N R V H F K S F N K D P 20

ttg gag ggc aag tct ggt gcc agc agc cgc ggt aat tcc agc tcc aat agc gta tat taa 120

L E G K S G A S S R G N S S S N S V Y - 39

agt tgt tgc ggt taa aaa gct cgt agt tgg ttc tgc gtc cca cgc tgt cgg ttc gcc gcc 180

S C C G - K A R S W F C V P R C R F A A 58

tgt cgg tgt aac tgg cat gtc gtg gca tgt cct gtc ggt ggt aaa cgg ggt ccc tgg tac 240
C R C N W H V V A C P V G G K R G P W Y 78
gac gta ggc ttt tat agc tga aat ctg tac cgt gtg tgt tcc cgt tta ccg atc tct cct 300
D V G F Y S - N L Y R V C S R L P I S P 97
act ccg gtg ctc tta aac gag tgt cga ggt agg ccg aca cgt tca ctt tga aca aat tag 360
T P V L L N E C R G R P T R S L - T N - 115
agt gct taa agc agg cta aaa tat ctg cct gaa tag tgg tgc atg gaa tga taa aac agg 420
S A - S R L K Y L P E - W C M E - - N R 131
acc tca gtt cta ttt tgt tgg ttt tag gaa tat gag gta atg atc aat gtg gac tgg cgg 480
T S V L F C W F - E Y E V M I N V D W R 150
ggg cat tcg tat tgc gac gtt aga ggt gaa att gtt gga tcg tcg caa gac gca cta gag 540
G H S Y C D V R G E I V G S S Q D A L E 170
cga aag cat ttg cca agt atg tct taa ttg atc aag aac gaa agt tag agg ttc gaa ggc 600
R K H L P S M S - L I K N E S - R F E G 188
gat cag ata ccg ccc tag ttc taa cca taa acg atg cca gcc agc gat ccg ccg atg ttc 660
D Q I P P - F - P - T M P A S D P P M F 205
gtt taa tga ctc ggc ggg gag ctt cta ctc ggg aaa cca aag ctt ttg ggt tcc ggg gga 720
V - - L G G E L L L G K P K L L G S G G 223
agt atg gtt gca aag ctg aaa ctt aaa gga att gac gga agg gca cca cca gga gtg gag 780
S M V A K L K L K G I D G R A P P G V E 243
cct gcg gct taa ttt gac tca cac ggg aaa ccc ccc cca aaa aaa a 826
P A A - F D S H G K P P P K K 257

Figure 1: The 826 bp nucleotide sequence and conceptually translated amino acid sequences of PCR amplicon of the 18S ribosomal RNA gene from *R.marginatus.*

Codon positions	**A**	**T**	**C**	**G**
First position	7.38	8.71	7.02	8.7
Second position	8.47	7.38	6.65	9.6

Third position	7.5*	9.07*	6.9	8.47

Table 1: Base Composition in the 826 bp nucleotide sequence of the 18S ribosomal RNA gene at the three codon positions in *R.marginatus*.

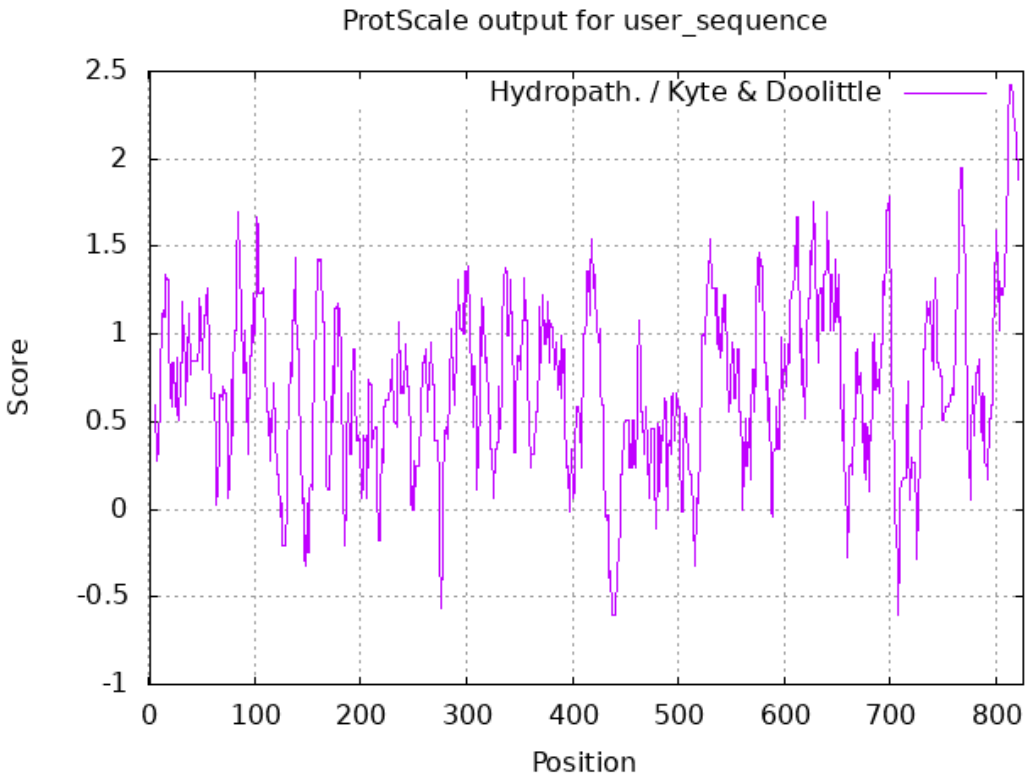

Figure 2: Hydropathy plot of the in-silico translated partial 18S ribosomal RNA gene protein from the 826 bp nucleotide sequence from *R.marginatus*

No.	Nucleotide sequence obtained (bp)	A	A%	T	T%	C	C%	G	G%	AT%	GC %
1	826	214	25%	212	28%	177	21%	223	26%	53%	47%

Table 2: Nucleotide composition of the partial sequenced 18Sribosomal gene from the *R.marginatus*.

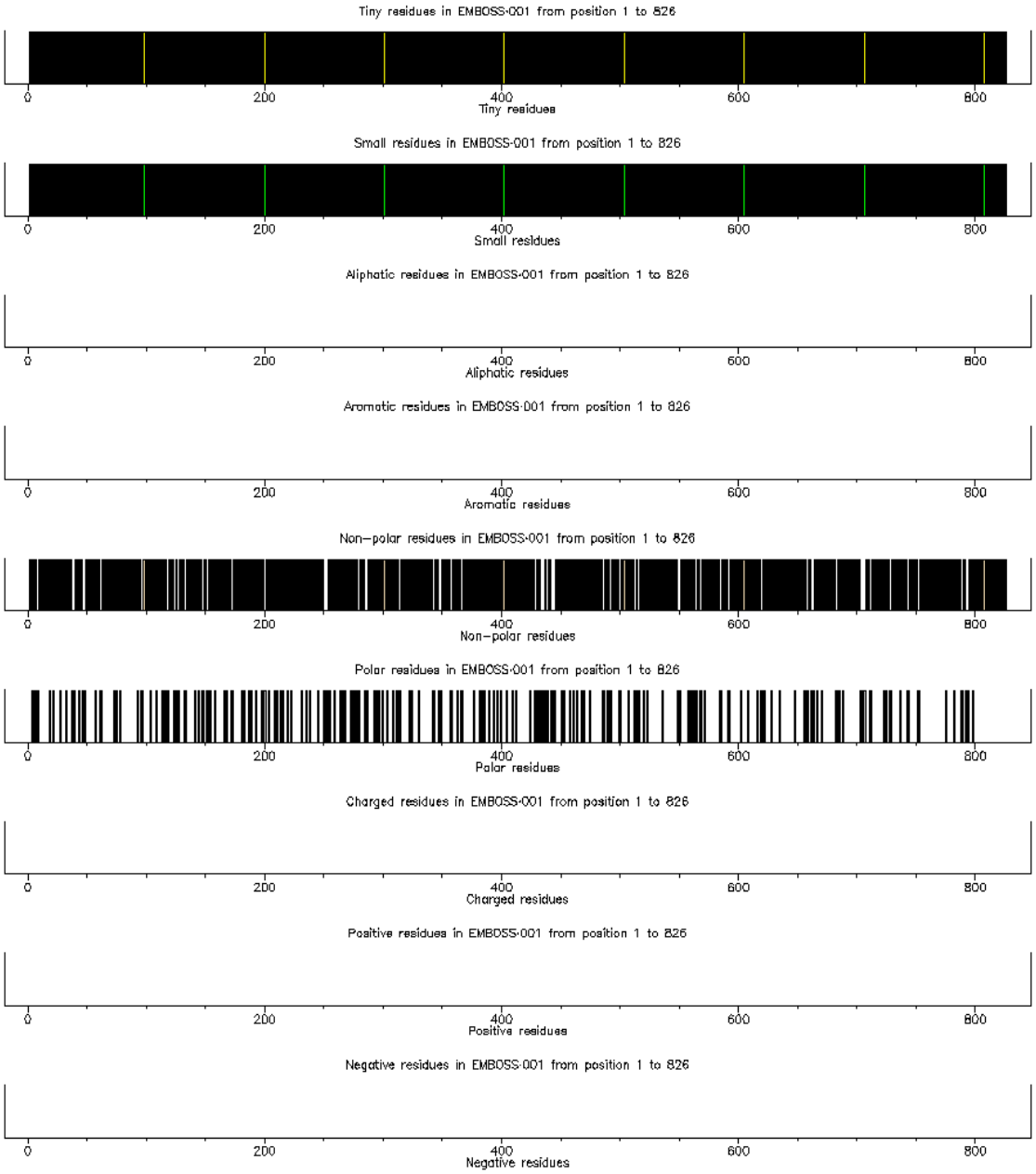

Figure 3: Histogram plot of the in-silico translated 826 bp nucleotide sequence of the 18S ribosomal RNA gene protein from *R. marginatus.*

Percentage identity

		18s	16s	28s		Gene name
1	18s		50.99	48.75	1	18S

Divergence

2	16s	3.64		55.41	2	16S
3	28s	2.64	2.18		3	28S

Table 3: Percentage identity and divergence of the partial nucleotide sequence of the 18S, 16S and 28S Ribosomal RNAgenes from *R. marginatus*

Figure 4: Phylogenetic relationships of the three genes (16S, 18S, 28S ribosomal RNA gene) of *R. marginatus* based on nucleotide sequence of the PCR amplicon of the Ribosomal genes derived from Neighbor Joining Algorithm using Clustal Omega (Software 1.2.4)

Discussion

In the present investigation a 826 bp of the gene amplicons were recorded for the *R. marginatus* insect. On sequencing the 18S gene sequenced region matched with the already reported 18S gene sequence of some of the insect species that falls under the family of reduviidae. The sequence of the 18S gene generated in this study matched with sequence information results that are already reported in otherinsects Jon *et al*., in Hansenilla>1970 nucleotides[11] and Chiristane*et al*., were studied about the assassin bugs in the same gene[12] and Yingqi*et al*., also reported in the same gene in the assassin bug *Sigicoris stat*[13], Uday kumar*et al*., reported in *Linguataula serrata* insect[14] and Gillespie *et al*., were reported on *Apis melifera*[15], Anil kumar*et al*., on *Theileria annulata*[16].

The nucleotide comparison and an amino acid sequences across the three ribosomal genes from *R. marginatus*indicated a higher divergence value of 3.64% and 2.64% in 18S and 16S genes respectively

than that other 28S gene from *R. marginatus*. In related work has done and reported by already in the same insect. In higher genetic divergence values have been Cyt b and COI genes from four *Rhynocoris*species. [5]

Analysis of the nucleotide sequence of the *R. marginatus* insect three Ribosomal genes are indicated higher nucleotide substitutions in 18S gene when compared to the other ribosomal genes 16S and 28S. Ambros *et al*., were studied and reported into intrageneic phylogenetic relationships between thirteen species of *Coranus Curtis*[2] and Eisuke *et al*., 2006 were reported the phylogenetic analysis of the insect order Odonata[17], Mahendran *et al*., were reported into *Bombycidae.*[18] And already reported in other insects such as, *Chironomus* (Diptera) species Guryev *et al*., [19] and Jon *et al.,* [11] , Yingqi*et al.,* in *Sigicoris*[13], Austin *et al.,* [20], Arunkumar *et al.,* [21] in *Bmbyxmor*i and yogesh*et al*., [22] also reported in similar gene in various insect orders.

Here already reported to the phylogenetic analysis of various genes in species level. Such as Jon *et al.,* were reported *Hansenilla* has analysis phylogenetic tree and topological studies[11] and Christane *et al*., 2009 were reported into Assassin bugs[12], Udhay kumar*et al.,* on *Linguatula serrata*[14], Yingqi*et al.,* were reported in phylogenetic analysis in assassin bug *Sigicoris* stat[13] and Gillespie *et al.,* 2006 were reported on *Apis melifera*[15], Anil kumar*et al*., 2022 on *Theileria annulata.*[16] And some relative studies were reported in the same species such as Baskar *et al.,* were reported the phylogenetic relationships between the *Rhynocoris* species in four *Rhynocoris*, like *R. marginatus, R.longiffrons, R.fuscipes* and *R. kumarri*.[5] Ambrose *et al*., reported in the genomic relationships among the four *harpatorine* reduviid species of *Rhynocoris;* like *R. kumarii*, *R. marginatus, R. Longifrons and R.fuscipes*. [2]

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 18Sribosomal RNA sequence in multiple and phylogenetic analysis. In ribosomal 18S gene of *R. marginatus,* population which can be used to develop molecular markers important for examining molecular genetic variation or gene diversity and understanding deep phylogenetic relationship the utility across the available heteropteran mtDNA ribosomal genome to facilitate informed gene choice for molecular study the *R. marginatus.*These results should allow the identification of the genetic variation and the analysis of phylogenetic information for understanding in *R. marginatus*genetic evolution.

References

1. Fritz G. N., Conn J., Cockburn A. S and Eawright J. Sequence analysis of ribosomal DNA internal transcribed spaces 2 from populations of *Anopheles nunezyovari* (Diptera: Culicidae). *Molecular Biology and evolution*.1994; 11: 406-416.

2. Ambrose E., Arockia l. and Angeline K. D. Intrageneric phylogenetics based on mitochondrial DNA variation among fifteen harpactorine assassin bugs with four ecotypes and three morphs (Hemiptera :Reduviidae: Harpactorinae). *Zootaxa.* 2014;3779 (5): 540-550.
3. Putshkov V. G., Putskov P.V. A catalogue of assassin bugs genera of the world (Heteroptera :Reduviidae). *VINITI, Moskva*. 1985; 138.
4. Maldonado J. Systematic catalogue of the Reduviidae of the world (Insecta :Heteroptera). Caribbean *Journal of Science special editions, University of Puerto Rico, Mayaguez.* 1990; 694.
5. Baskar A., Dhanasekaran K. G., Thirumurugan A. R., Vignesh and Prakash. B. Molecular genetic variation and phylogeny of genus Rhynocoris based Mitochondrial Cytochrome c Oxidase subunit I gene (Heteroptera: Reduviidae). *Indian journal of Natural Sciences.* 2014; 4(22): 1-13.
6. Wolstenholme. Animal mitochondrial DNA structure and evolution. *Int Rev. cyto.*1992.
7. Yu Nie, Yu- Thian Fu. Yu Zhang Yun ping Deng, Wei wang, Ya Tu and Guo –Hau liu., 2021. Highly rearranged mitochondrial genome in falcoliperurislice (Phthiroptera; Philoptera) from endangered eagles.
8. Avise J. C., Arnold J., Ball R. M., Berminghan E., Lamb T., Neigel J., Neigel J. E., Reeb C. A., Saunder N. C., 1987. Intraspecific phylogeograpy the mitochondrial DNA bridge between population genetics and systematic. *Annu, Rev.Ecol.syst*. 1987; 18: 489-522.
9. Moritz C., Dowling T. E., Brown W. M. Evolution of Animal mitochondrial DNA relevance for population biology and systematic. *Annu, Rev. Ecol. Syst.*1987; 269-292.
10. Arthur K., Maria K., Emeline I., Eric C., Julie P., Jerome C., Jerome M. Shot gun assembly of the assassin bug Brontostoma colossus mitochondrial genome (Heteroptera: Reduviidae). *Gene*. 2014; 4c: 39959-11.
11. Jon M. M., James R., Garey. and Jeffrey. W. S. Ecdysozoan phylogeny and Bayesian inference: First use of nearly complete 28s and 18s rRNA gene sequences to classify the arthropods and their kin. *Molecular phylogenetic and evolution*. 2003. 5: 213-216.
12. Christians W., James B Munro. 2009. Molecular phylogeny of the assassin bugs (Hemiptera: Reduviidae), based on mitochondrial and nuclear ribosomal genes. *Molecular phylogenetics & Evolution.* 2009; 53, 287-299.
13. Yingqi L., Hu L. and Wanzhi C. Revision of the Assassin Bug geneus*Sigicoris Stat* Nov Based on morphological study and molecular phylogeny (Heteroptera: Reduviidae: Peiraatinae). *Insect*-2022; 13951.
14. Uday kumar M. and Tasashi I. T. Molecular characterization and phylogeny of *Linguatala serrata* (Pentastomida :Linguatulidae) based on the nuclear 18s rDNA and mitochondrial cytochrome C oxidase I gene. *J. vet. Med . Sci.* 2017; 79 (2): 398-402.

15. Gillespie, J. J., Johntoon, J. S., Cannones J. J., and Guttells R. R. Characteristics of the nuclear (18s, 5.8s, 28s and 5s) and mitochondrial (12s and 5s) rRNA genes of *Apis mellifera* (Insecta: Hymenoptera) structure, organization, and retro transposable elements. *Insect molecular biology.* 2006; 15 (5): 657-686.
16. Anilkumar N., Ansu Kumari V. R., Kundave Sukhdeep V., Hira R.Molecular insights into the population structure and haplotype network of *Therilaannulata* based on the small sub unit ribosomal RNA (18S rRNA) gene. *Infection, Genetics and Evolution.* 2022; 99: 105252.
17. Eisuke H., and Eiiti K. Phylogenetic analysis of the insect order odonata using 28s and 16s rDNA sequence; a comparison between data sets with different evolutionary rates. *Entomological science*. 2006; 9: 55-66.
18. Mahendran B., Ghosh S. K and Kundu S. C. Molecular phylogeny of silk producing insects based on 16s ribosomal RNA and Cytochrome oxidase subunit I gene. Indian Acadamy of science. *J. Genet.* 2006; 85: 31-38.
19. Guryev V., Makarevitch I., Blinov A. and Martin J. Phylogeny of genes Chirnomous (Diptera) inferred from DNA sequences of mitochondrial Cytochrome b and Cytochrome oxidase I. *Mol. Phylogenet. Evol*. 2001; 19: 9-21.
20. Austin J. W., Szalanski Allen L., and Kard Bradford M. Distribution and genetic variation of Reticlatitermes (Isoptera: Rhinotermitidae) in Oklahoma. *Florid Entomological Society.* 2004; 87(2):152-158.
21. Arunkumar Muralidhar Melta K. P., Nagaraja J. Molecular Phylogeny of silkmoths reveals the origin of domesticated silkmoth, *Bombyx mori* from Chinese *Bombyx mandarina* and paternal inheritance of *Antheraea proylei* mitochondrial DNA. *Molecular phylogenetics and Evolution*. 2006; 40: 419-427.
22. Yogesh S., Shouche and Milind S patole. Sequence analysis of mitochondrial 16s ribosomal RNA gene fragment from seven mosquito species. *J. Bio Sci.* 2000; 25: 361-366.