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Composition characteristics of the Mitochondrial encoded *COX1* gene of Triatominae (Heteroptera: Reduviidae)

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ABSTRACT: Triatominae is the only hematophagous subfamily of Reduviidae. They are vectors of *Trypanosoma cruzi* which causes Chagas' disease. Complete mitochondrial genomes provide reliable phylogenetic information from its highly conserved protein-coding genes including *MT-COX1*, which is widely used in molecular phylogenetics. This study investigated the compositional characteristics of the *MT-COX1* gene of 11 Triatominae species to detect the variations/patterns which support phylogenetic systematics. Curated mitogenomes of various species in *Triatoma* are more in Refseq than other genus in Reduviidae. The AT biased nucleotide composition showed negative AT and GC skewness. Leucine and isoleucine dominate the amino acid composition. When comparing the *MT-COX1* coding gene among the 11 species, 83.45 ± 2.90% genetic identity with heat map analysis showing the divergence among the tribes. Amino acids with hydrophobic side-chains (especially Leu and Ile) are subjected to conservative substitutions. Non-conservative substitutions are more favored than semi-conserved mutations. An 86.49% threshold of identical amino acids in the *MT-COX1* is prominent within the subfamily. This study encountered challenges in delineating validated nucleotide sequences, analyzing them within the framework of established morphological systematics and elucidating the mechanisms underlying molecular evolution within the gene. Thus, studying the basic compositional variations in the protein-coding genes like *MT-COX1* substantiate mechanisms of evolution in Triatominae. The highly variable amino acid sequence from 472 to 492 of *MT-COX1* and corresponding coding region in the gene can delimit species in Triatominae. Gaining insight into the fundamental compositional variations within dependable protein-coding genes, such as MT-COX1, not only enhances the validation of classical taxonomy through molecular systematics but also reveals contrariety in the phylogenetic relationship of Triatominae, extending up to the level of Tribes.

Keywords: Insect vectors, Hematophagy, Mitochondria, Amino acid, Systematics, Phylogeny.

INTRODUCTION

Insects are both beneficial and harmful to humans. From being ideal models for fundamental biological research to notorious pests and vectors, the study of insects is unavoidable. One such harmful insect are Triatomine bugs also known as the kissing bugs. The family Reduviidae consists of terrestrial heteropterans in which the subfamily Triatominae are exclusively hematophagous while the others are predators. They have long snout-like ante ocular region (Conenose) with a thin and straight rostrum which differentiate this subfamily from other predatory Reduviidae (Ambrose, 1999 and 2017). There are more than 150 species under 18 genera and 5 tribes *viz*., Alberproseniini, Bolboderini, Cavernicolini, Rhodniini and Triatomini (Zhao *et al.*, 2021). They are mainly distributed across North and South America. The genus *Linshcosteus* found in the Indian subcontinent and *Triatoma rubrofasciata* over South-east Asia to New Guinea up to Northern Australia (Otálora-Luna *et al.*, 2015). These bugs are nocturnal, feeding on vertebrate blood living in association with their hosts in sylvan dwellings such as palm trees, bird nests, rock crevices (Patterson *et al.*, 2001; Sandoval *et al.*, 2013) and thatched roofs (Schuh and Weirauch 2020). They transmit the protozoan *Trypanaosoma cruzi* which causes Chagas' Disease (American Trypanosomiasis) through their feces while feeding. This zoonotic disease is one of the most overlooked tropical diseases affecting an estimated 6-7 million people in Latin America (Ambrose, 2017; WHO, 2023). The mitogenome of most animals is highly conserved (Burger *et al.*, 2003). The circular mitochondrial genome of insects comprises 13 protein-

coding genes, 22 tRNAs, 2 rRNAs and a control region (Cameron, 2014a). Complete mitochondrial genomes provide reliable phylogenetic information using features such as protein-coding gene mutations, gene rearrangements, nucleotide composition and organization of the control region (Wang, 2015). There are 5 protein complexes involved in the oxidative phosphorylation pathway. Except the protein complex II which is encoded in the nucleus (genomic DNA), the protein complexes I, III, IV and V are encoded in the mitochondrial genome. The three genes of complex IV are *COX1*, *COX2* and *COX3* (Cuperfain *et al.,* 2018). *COX1* is so reliable that it is used primarily in DNA Barcoding of both plants and animals (Jung *et al.*, 2011; Kabiraj *et al.,* 2022). The effectiveness of the COX1 gene in species identification was evaluated, and the obtained results consistently demonstrated a high level of genetic diversity. Additionally, a notable gene flow was observed among the studied populations, indicating a lack of isolation by distance across different geographic regions. The accuracy of DNA barcoding analysis in distinguishing specimens from other species was demonstrated, achieving a 100% identification precision (Maia *et al*., 2015; Choudhary *et al.*, 2016 and 2017). It is worth noting that previous studies primarily utilized complete mitochondrial genomes, lacking the exploration of fundamental compositional variations within reliable protein-coding genes and the analysis of such genes within established morphological systematics. The mechanisms underlying molecular evolution within the gene were not elucidated in those studies (Pita *et al.*, 2017; Zhao *et al*., 2019; Aguilera-Uribe *et al.*, 2020; Tian *et al.*, 2022; Lian *et al.*, 2022). Hence, this study investigates the compositional characteristics of the *MT-COX1* gene of 11 Triatominae species to detect the variations which support molecular systematics and understand the mechanism of evolution within the subfamily.

METHODS

1. Data collection and processing: The complete mitochondrial genomes of Triatominae catalogued in the National Center for Biotechnology Information (NCBI) Refseq database (https://www.ncbi.nlm.nih.gov/refseq) were chosen (Table 1) and downloaded from the GenBank as FASTA (.fasta) files. The 13 protein-coding gene sequences and their translated amino acid sequences were generated using Unipro UGENE software v44.0 (https://ugene.net/).

2. Analysis: Multiple sequence alignment was computed in MAFFT 7 online server (https://mafft.cbrc.jp/) using default parameters. The nucleotide composition, amino acid composition and codon usage of the gene were computed using MEGA XI software (https://www.megasoftware.net). Strand asymmetry was calculated using the formulae AT-skew = [A − T]/[A + T] and GC-skew = [G − C]/[G + C]. The results were presented using Numbers v12 for Mac. Genetic identity (%) and identical residues were determined using the 'Ident and Sim' tool from the sequence manipulation suite (https://www.bioinformatics.uni-muenster.de/tools/sms2/). A single cluster euclidean distance method heat map was created with the genetic identity (%) between the selected species using SRplot (https://www.bioinformatics.com.cn/srplot).

Table 1: Triatominae mitogenomes selected for the study.

Tribe	Species	Abbreviation	Gen Bank Accession No.
Triatomini	*Triatoma migrans*	*Tmig/TT1*	NC_042881.1
	Triatoma infestans	*Tinf/TT2*	NC_035547.1
	Triatoma dimidiata	*Tdim/TT3*	NC_002609.1
	Triatoma lecticularia	*Tlec/TT4*	NC_050326.1
	Triatoma mazzottii	*Tmaz/TT5*	NC_050327.1
	Triatoma hueheutenanguensis	*Thue/TT6*	NC_050325.1
	Triatoma mexicana	*Tmex/TT7*	NC_050324.1
	Triatoma sanguisuga	*Tsan/TT8*	NC_050329.1
	Panstrongylusrufotuberculatus	*Pruf/TT9*	NC_042682.1
Rhodniini	*Rhodniuspictipes*	*Rpic/RH1*	NC_043846.1
	Rhodniusprolixus	*Rpro/RH2*	NC_050328.1

RESULTS AND DISCUSSION

1. Species selected for the study: Reference sequence records are only used in the study, as they are reviewed and curated by the NCBI staff from sequences published by the authors in the NCBI GenBank. Therefore, using these validated sequences becomes helpful in standardization of the analysis. Among the 11 species selected for the study (Table 1), 9 belong to the tribe Triatomini (8 *Triatoma* and 1 *Panstrongylus*) and 2 to Rhodniini (*Rhodnius*). Genus *Triatoma* of the tribe Triatomini is the most abundant genera of Triatominae (Justi and Galvão 2017). This is reflected in the diversity of the mitochondrial genomes available in NCBI GenBank Refseq as the genus *Triatoma* is the only genus of Reduviidae with the complete mitochondrial genomes of 8 species (https://www.ncbi.nlm.nih.gov/refseq).

Table 2: Nucleotide composition (%) and skewness of the *MT-COX1* gene of Triatominae.

Species	Length (bp)	A	T	G	C	A+T	G+C	AT skew	GC skew
Tmig	1534	31.10	34.09	15.65	19.17	65.19	34.81	-0.05	-0.10
Tinf	1534	30.12	31.62	16.23	22.03	61.73	38.27	-0.02	-0.15
Tdim	1534	30.18	32.66	16.36	20.80	62.84	37.16	-0.04	-0.12
Tlec	1534	29.73	33.77	16.75	19.75	63.49	36.51	-0.06	-0.08
Tmaz	1534	30.18	33.90	16.10	19.82	64.08	35.92	-0.06	-0.10
Thue	1534	30.77	33.31	15.84	20.08	64.08	35.92	-0.04	-0.12
Tmex	1534	30.57	32.92	16.30	20.21	63.49	36.51	-0.04	-0.11
Tsan	1534	29.86	32.72	16.82	20.60	62.58	37.42	-0.05	-0.10
Pruf	1534	30.38	30.70	16.82	22.10	61.08	38.92	-0.01	-0.14
Rpic	1534	31.68	33.44	15.12	19.75	65.12	34.88	-0.03	-0.13
Rpro	1536	31.90	30.40	15.89	21.81	62.30	37.70	0.02	-0.16
Mean	1534	30.59	32.69	16.17	20.56	63.27	36.73	-0.03	-0.12

2. Length and Nucleotide composition: The length of the *MT-COX1* is 1,534 bp except *R. prolixus* which is 1,536 bp (Table 2).

The nucleotide count of in the *MT-COX1* of the 11 species of Triatominae is illustrated in Fig. 1, where adenine range from 456 (*T. lecticularia*) to 490 (*R. prolixus*), thymine at 467 (*R. prolixus*) to 523 (*T. migrans*), guanine at 232 (*R. pictipes*) to 258 (*T. sanguisuga* and *P. rufotuberculates*) and cytosine with 294 (*T. migrans*) to 339 (*P. rufotuberculates*). The average nucleotide composition of A, T and A+T are 30.59%, 32.69% and 63.27% respectively. For nucleotides G, C and G+C are 16.17%, 20.56% and 36.73%. Strand asymmetry was found to be negative for both AT and GC with an average of -0.03 and -0.12 respectively. Positive AT skewness (0.02) was observed only with *R. prolixus* (Table 2). AT bias is common in invertebrate mitochondrial genomes including insects (Cameron, 2014b).

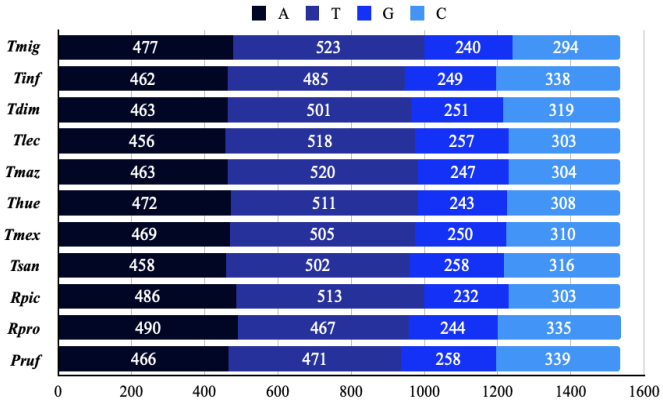

Fig. 1. Nucleotide residues in the the*MT-COX1* gene of Triatominae.

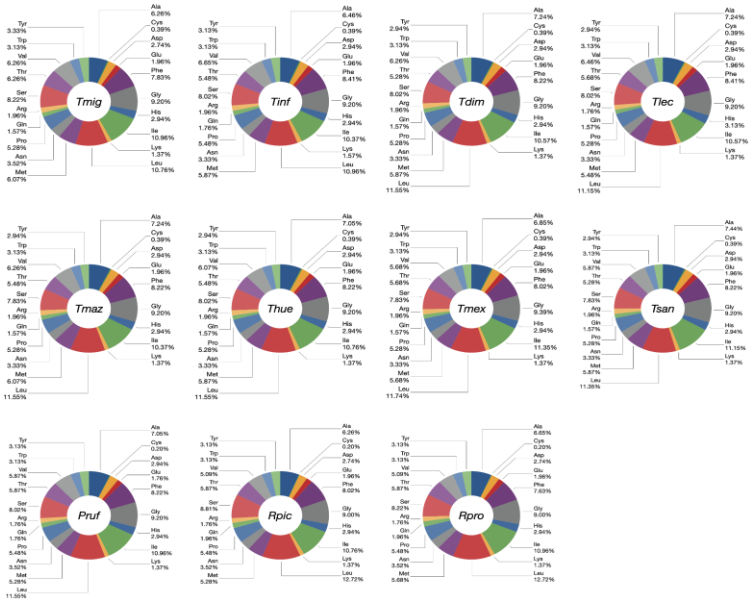

Fig. 2. Amino acid composition of the *MT-COX1* gene of Triatominae. Species names in the donut abbreviated as described in Table 1.

3. Amino acid composition and codon usage: The quantity of the amino acids present in the *MT-COX1* gene in the 11 Triatominae species are in the order Leu (10.76 - 12.72%) > Ile (10.37 - 11.35%) >Gly (9.00 - 9.39%) > Ser (8.02 - 8.81%) >Phe (7.63 - 8.41%) > Ala (6.25 - 7.44%) > Val (5.09 - 6.65%) >Thr (5.28 - 6.26%) > Met (5.28 - 6.07%) > Pro (5.28 - 5.48%) >Asn (3.33 - 3.52%) > Tyr (2.94 - 3.33%) >Trp (3.13%) > His (2.94 - 3.13%) > Asp (2.74 - 2.94%) > Arg (1.76 - 1.96%) > Glu (1.76 - 1.96%) > Gln (1.57 - 1.96%) > Lys (1.37 - 1.57%) >Cys (0.20 - 0.39%) as illustrated in Figure 2. Figure 3 shows the codon usage of the *MT-COX1* gene of the 11 Triatominae species (The invertebrate mitochondrial code - transl_table=5). The amino acid Leucine is encoded mostly by UUA followed by residues fluctuating between CUU and CUA across the 11 Triatominae species. Isoleucine is encoded by AUU > AUC. GGA > GGU > GGG > GGC with Glycine. Serine has 8 codons with the majority encoded by UCA and AGA but lack AGG. UUU and UUC encoding Phenylalanine vary between the species. GCG of Alanine is lacking in most species. GUA of valine and ACA of Threonine dominate with ACG is encoded only in *P. rufotuberculates* and *R. prolixus.* AUA is more prevalent in Methionine. In the two *Rhodnius* species CCC and CCA for proline are identical. Except *T. infestans* AAU > AAC with Asparagine and UAU and UAC compensated with encoding Tyrosine. Tryptophan is predominated by UGA with *T. infestans*, *R. pictipes* and *R. prolixus* lacking UGG. Like Tyrosine the codons CAU and CAC compensate. CGA dominates encoding Arginine where CGG is seldom. Clear prevalence of GAA with Glutamic acid, CAA with Glutamine and AAA with Lysine is seen. UGU and UGC encode Cysteine either single or in combination (in case of 2 residues). The strict lack of AGG in Serine is common in insect mitochondrial genomes as reported earlier in hemipterans (Wang, 2015) and other Triatominae species (Zhao *et al*., 2019).

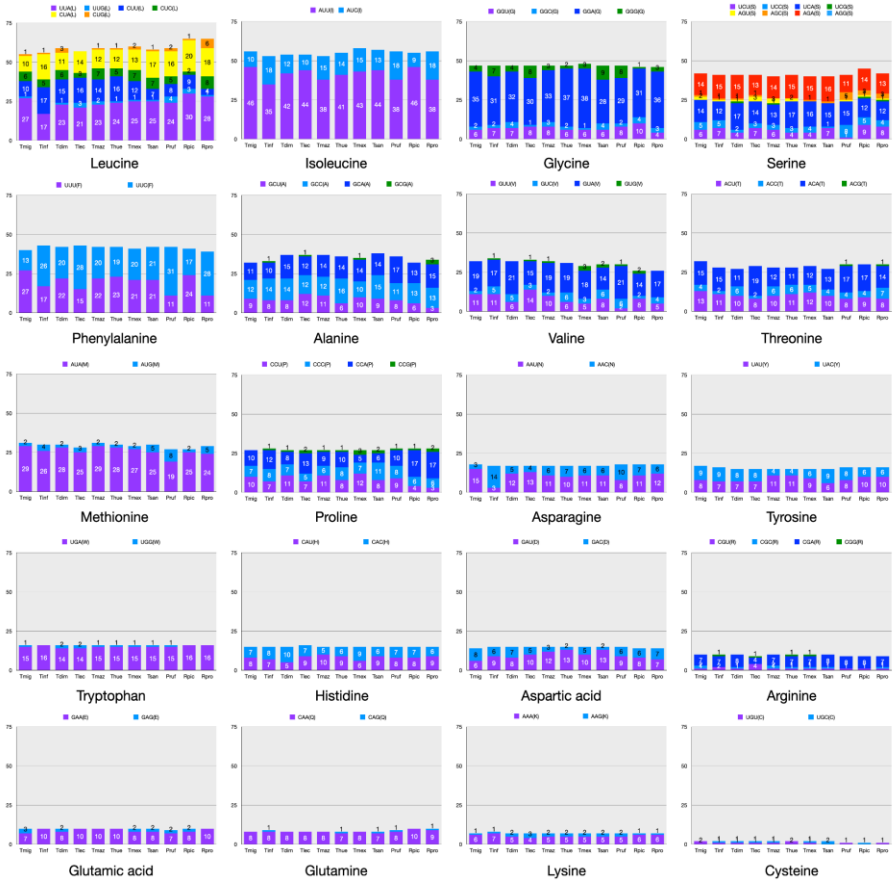

Fig. 3. Codon usage of the *MT-COX1* gene of Triatominae (refer Table 1 for species name abbreviations).

4. Comparative analysis: Table 3 shows that the genetic identity (%) of the *MT-COX1* between the 11 species of Triatominae is 83.45 ± 2.90 (Mean ± S.D.) with 1280 ± 44 (Mean ± S.D.) identical residues. The maximum identity was observed between *T. dimidiate* and *T. mazzotii* with 90.16% and 1,383 identical residues. *T. Mexicana* and *R. prolixus* show least identity (79.43% and 1,220 residues). Percentage identity and identical residue values 22/55 (40%) of the comparison between the 11 species is greater than average implying noticeable interspecific variations. Heat map analysis (Fig. 4) reveal that the species within a tribe are more similar and conform to the phylogenetic analysis performed from earlier studies using mitochondrial genomes of Triatominae (Dotson and Beard 2001; Pita *et al.*, 2017; Zhao *et al.*, 2019; Aguilera-Uribe *et al*., 2020).

Table 3: Genetic identity of the *MT-COX1* gene of Triatominae.

	Tmig	***Tinf***	***Tdim***	***Tlec***	***Tmaz***	***Thue***	***Tmex***	***Tsan***	***Rpic***	***Rpro***	***Pruf***
Tmig		82.07	84.22	84.09	84.62	84.81	83.70	83.31	81.88	79.75	82.86
Tinf	1259		82.99	82.07	83.57	81.62	82.01	82.66	81.03	79.95	82.40
Tdim	1292	1273		85.66	90.16	89.90	88.85	88.27	80.31	80.47	82.79
Tlec	1290	1259	1314		85.07	84.49	84.49	85.07	80.64	81.05	82.66
Tmaz	1298	1282	1383	1305		89.31	88.72	86.77	80.57	80.53	82.53
Thue	1301	1252	1379	1296	1370		88.07	87.16	80.83	80.53	82.59
Tmex	1284	1258	1363	1296	1361	1351		87.48	80.64	79.43	82.14
Tsan	1278	1268	1354	1305	1331	1337	1342		82.27	80.60	82.86
Rpic	1256	1243	1232	1237	1236	1240	1237	1262		86.26	80.44
Rpro	1225	1228	1236	1245	1237	1237	1220	1238	1325		80.73
Pruf	1271	1264	1270	1268	1266	1267	1260	1271	1234	1240	

Footnote: Upper right - Percentage identity (Mean ± S.D. = 83.45 ± 2.90); Lower left - Identical residues (Mean ± S.D. = 1280 ± 44)

5. Conserved amino acid residues: Length of the *MT-COX1* protein sequence is highly conserved with 511 residues. ATG is the start codon found in *MT-COX1* gene of Triatominae and 9/11 species selected for the study terminate with incomplete T (Cameron, 2014b) except *R. prolixus* having TAG (The invertebrate mitochondrial code transl_table =5).

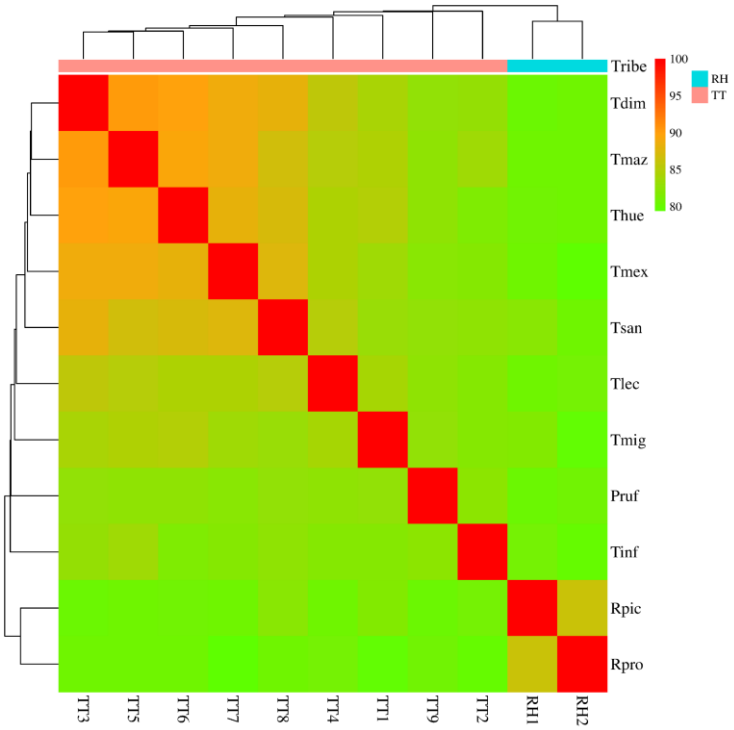

Fig. 4. Heat map of the similarity in *MT-COX1* gene of Triatominae. Species names in the map abbreviated as described in Table 1.

Form the Fig. 5, 442/511 (86.49%) residues are highly conserved i.e., identical, 51/511 (9.98%) residues are similar (conserved mutation), 5/511 (0.97%) residues have semi-conserved mutation and 13/511 (2.54%) residues have non-conservative mutations (dissimilar amino acids). Most conserved substitutions are from amino acids with hydrophobic side-chains (A, V, I, L, M, F except Y and W which are conserved). Non-conservative substitutions are more favoured than semi-conserved mutations. Natural selection mainly functions at the protein-level hence, studying the substitution rates in protein-coding genes can unveil progress of natural selection (Álvarez-Carretero *et al.*, 2023).

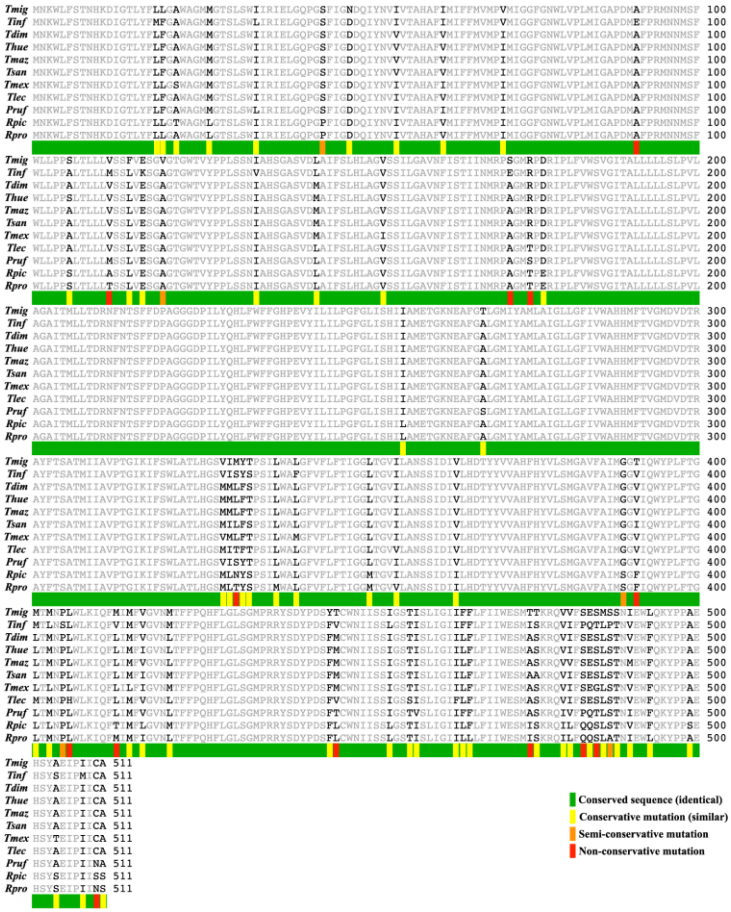

Fig. 5. Conserved amino acid residues in the *MT-COX1* of Triatominae (note: Illustrated colours correspond to multiple sequence alignment symbols '*'- green, ':' - yellow, '.'- orange and '<space>' - red respectively).

CONCLUSIONS

The amino acid sequence from 472 to 492 identified variation among the species in *Triatoma.* Hence, the corresponding coding region of 472 to 492 amino acids in the gene can delimit species in Triatominae (Fig. 5). Future studies are needed to compare this region with other species in Triatominae. When comparing all the results of this study, leucine and isoleucine, being the most abundant amino acids, are subjected to conservative mutations. A threshold of 86.49% identical amino acids and a very close 83.45 ± 2.90 (Mean ± S.D.) genetic identity (%) is prominent within the subfamily.

FUTURE SCOPE

Therefore, understanding the basic compositional variations in the reliable protein-coding genes like *MT-COX1* supplements the validation of classical taxonomy through molecular systematics. Further investigations with all the MT-genes can disclose contrariety in the phylogenetic relationship of Triatominae.

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Conflict of Interest. None.

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